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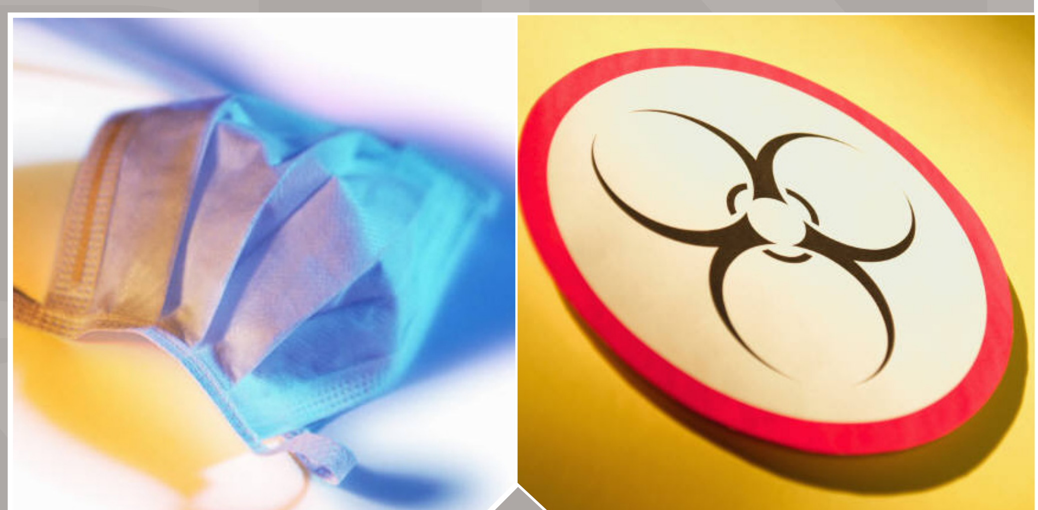
Persistence Testing and Evaluation of Fumigation Technologies for Decontamination of Building Materials Contaminated with Biological Agents



Office of Research and Development
National Homeland Security Research Center

Persistence Testing and Evaluation of Fumigation Technologies for Decontamination of Building Materials Contaminated with Biological Agents

SHAWN P. RYAN
NATIONAL HOMELAND SECURITY RESEARCH CENTER
OFFICE OF RESEARCH AND DEVELOPMENT
U.S. ENVIRONMENTAL PROTECTION AGENCY
MAIL CODE E343-06
RESEARCH TRIANGLE PARK, NC 27711

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Questions concerning this document or its application should be addressed to:

Shawn P. Ryan
National Homeland Security Research Center
Office of Research and Development
U.S. Environmental Protection Agency
Mail Code E343-06
Research Triangle Park, NC 27711
919-541-0699
ryan.shawn@epa.gov

If you have difficulty assessing these PDF documents, please contact Nickel.Kathy@epa.gov or McCall.Amelia@epa.gov for assistance.

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M. Worth Calfee

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Timothy Dean

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R. Leroy Mickelsen

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Foreword

The Environmental Protection Agency (EPA) hold responsibilities associated with homeland security events: EPA is the primary federal agency responsible for the country's water supplies and for decontamination following a chemical, biological, and/or radiological (CBR) attack. The National Homeland Security Research Center (NHSRC) was established to conduct research and deliver scientific products that improve the capability of the Agency to carry out these responsibilities.

An important goal of NHSRC's research is to develop and deliver information on decontamination methods and technologies to clean up CBR contamination. The research described here provides specific information that will aid EPA and other stakeholders in choosing a decontamination method or technology when addressing clean up of biological threat agents. NHSRC evaluated the effectiveness of several fumigation technologies against a number of agents under various environmental conditions. In addition, this document presents information on persistence of the agents in the absence of fumigation to help assess the feasibility of clean up by natural processes.

NHSRC is pleased to make this publication available to assist the response community prepare for and recover from disasters involving CBR contamination. This research is intended to move EPA one step closer to achieving its homeland security goals and its overall mission of protecting human health and the environment while providing sustainable solutions to our environmental problems.

—Gregory Sayles, Ph.D., Acting Director
National Homeland Security Research Center

Executive Summary

The U.S. Environmental Protection Agency's National Homeland Security Research Center (NHSRC) is helping to protect human health and the environment from adverse impacts resulting from acts of terror by identifying methods and equipment that can be used for decontamination following a terrorist attack in which chemical, biological, or radiological agents are used and by investigating the fate (e.g., persistence) of such agents in the absence of decontamination. The persistence of biological agents is influenced by environmental conditions and the materials with which the biological agents are in contact. The generation of scientifically defensible persistence data is useful for the proper planning of decontamination efficacy tests and helps formulate first response plans in preparation for possible natural occurrences or intentional releases of biological agents. In this current effort, persistence data were generated for *Brucella suis*, *Francisella tularensis*, vaccinia virus (a surrogate for the variola virus that causes smallpox), and *Yersinia pestis*. Additionally, four fumigation technologies (Sabre chlorine dioxide [ClO_2], BIOQUELL Clarus C HP [hydrogen peroxide], BIOQUELL Clarus S HP, and STERIS VHP® [Vaporized Hydrogen Peroxide]) were evaluated for their ability to decontaminate several materials contaminated with an array of biological agents, including *Bacillus anthracis* spores, *B. suis*, *F. tularensis*, vaccinia virus, or *Y. pestis*.

The intent of the fumigant testing was to assess the ability of the technology or decontamination process to decontaminant materials at conditions consistent with use in a facility. However, laboratory testing may present a challenge when testing at a smaller scale than for which the decontamination equipment was designed. For the Sabre ClO_2 testing, Sabre Technical Service, LLC. provided a prototype unit designed for reproducing their process in a smaller, lab-scale, environment (e.g., 317 L glove box). For the BIOQUELL hydrogen peroxide fumgition, the initial intent was to test using the 317 L glove box. In order to represent a typical room fumigation with the BIOQUELL hydrogen peroxide fumigation process, the temperature rise in the enclosed space due to the fumigation equipment must be minimized. To accomplish this in lab testing, BIOQUELL provided their Clarus S unit desgined for typical use in biological safety cabinets. After testing with that unit, it was decided to test at a larger scale (1275 L Biological Safety Cabinet), utilizing one of their larger fumigation units (Clarus C) with an attempt to obtain a better representation of room-scale fumigation. The STERIS VHP® system was a unit of similar size and design parameters to the BIOQUELL Clarus C, and tested at the same scale (1275 L).

Persistence

Persistence (recovery of viable organisms) was determined for *B. suis, F. tularensis*, vaccinia virus, and *Y. pestis* spiked onto four materials (aluminum, keyboard [computer keyboard keys], carpet, and joint tape [painted paper joint tape]) and held under ambient environmental conditions for up to 7 days. Persistence was determined by the recovery of biological agents from the materials at the completion of the exposure duration. The longest exposure duration for the biological agents recovered from the various materials was 7 days with the following exceptions (the longest durations yielding viable agent are noted in parentheses): *B. suis* on joint tape (4 hours [hr]), *F. tularensis* on aluminum and joint tape (8 hr) and carpet (4 hr), vaccinia virus on joint tape (3 days), and *Y. pestis* on keyboard (3 days) and carpet (8 hr) (see also Table ES-1).

Table ES-1. Longest Persistence Observed*

Biological Agent	Longest Duration with Viable Biological Agent Recovered by Material			
	Aluminum	**Keyboard**	**Carpet**	**Joint Tape**
B. suis	7 days	7 days	7 days	4 hr
F. tularensis	8 hr	7 days	4 hr	8 hr
Vaccinia Virus	7 days	7 days	7 days	3 days
Y. pestis	7 days	3 days	8 hr	7 days

* Testing was conducted for a maximum of 7 days.

Sabre ClO_2

The evaluation of Sabre ClO_2 was conducted with 3,000 parts per million by volume (ppmv) ClO_2 at both 40% and 75% relative humidity (RH) against *B. anthracis* spores. A lower ClO_2 concentration (50-100 ppmv) was used for the other biological agents (*B. suis*, *F. tularensis*, vaccinia virus, and *Y. pestis*), which were also tested at 40% and 75% RH. Fumigation was also conducted at 60% RH with *B. suis* and vaccinia virus. All tests were conducted with aluminum, keyboard, carpet, and joint tape at 23 °C ± 2 °C and contact times ranged from 0 to 180 minutes (min).

Following 180 min of exposure to 3,000 ppmv ClO_2, viable *B. anthracis* spores were not recovered from keyboard or carpet, but *B. anthracis* spores were recovered from aluminum (at 40% RH and 75% RH). *B. anthracis* spores were recovered from joint tape after the 180-min exposure at 40% RH but not after the 180-min exposure at 75% RH.

B. suis was not recovered from carpet or joint tape exposed to 50-100 ppmv ClO_2 (23 °C) for 120 min at 40% RH, 60% RH, or 75% RH. However, *B. suis* generally persisted on aluminum and keyboard. Only after 60-min or 120-min exposures to 50-100 ppmv ClO_2 at 75% RH was *B. suis* not recovered from aluminum. Interestingly, *B. anthracis* spores were most easily decontaminated from keyboard, but *B. suis* was most resistant to decontamination from keyboard.

F. tularensis was not recovered from any of the four test materials after 120-min exposure to 50-100 ppmv ClO_2 (23 °C and 75% RH). When tested at a lower RH (40%), *F. tularensis* was always recovered from aluminum and keyboard, but not from carpet or joint tape. (*F. tularensis* was not recovered from the carpet and joint tape positive controls associated with the contact times tested at the lower RH [40%]. Natural decontamination of *F. tularensis*, as spiked onto the materials, may occur over time.)

Vaccinia virus was recovered from all four materials following 120-min exposure to 50-100 ppmv at 23 °C and 40% RH. At 75% RH vaccinia was not recovered from aluminum, carpet, or joint tape after 30-min exposure to 50-100 ppmv ClO_2. Vaccinia virus persisted on keyboard even following a 120-min exposure to 50-100 ppmv ClO_2 at 75% RH.

Y. pestis was not recovered from any of the four materials tested when exposed to 50-100 ppmv ClO_2 at 40% RH or 75% RH. Positive controls show high levels of loss of viable *Y. pestis* without fumigation.

BIOQUELL Clarus C HP

The BIOQUELL Clarus C HP fumigation involved exposure to HP generated using cycle parameters (dehumidification, gassing phase, and dwell phase) specified by the manufacturer. The evaluation was primarily conducted with the following cycle: fumigate 10 min at 8 g/min and dwell at 0.8 g/min for contact times of 180 min. Tests were conducted on aluminum, keyboard, carpet, and joint tape with *B. suis*, *Y. pestis*, and vaccinia virus. Testing was also conducted with vaccinia virus on glass and with *B. anthracis* spores on carpet, laminate, ductwork, concrete, wood, glass, and ceiling tile. Some level of efficacy was observed against all types of biological agents on all surfaces after the 180-min contact time.

No viable *B. anthracis* spores were recovered from laminate, ductwork, ceiling tile, or glass. Viable *B. anthracis* spores were recovered from carpet, concrete, and wood after the 180-min exposure.

No viable *B. suis*, vaccinia virus, or *Y. pestis* was recovered from any of the materials tested following exposure to BIOQUELL Clarus C HP fumigation. Neither *B. suis,* vaccinia virus, nor *Y. pestis* was recovered from aluminum, keyboard, carpet, joint tape, or glass (used for vaccinia virus only) following a 180-min exposure to HP.

BIOQUELL Clarus S HP

The BIOQUELL Clarus S HP fumigation involved exposure of biological agents to various HP fumigation parameters (e.g., HP volume of 15 mL to 50 mL over injection times of 15 to 20 min) and contact times of 15 to 192 min. Testing was generally conducted at ambient temperature (22 °C) under two initial RH conditions (40% - 50% and 60% - 70%). Testing with *B. anthracis* spores was conducted only at 45% RH. Testing was conducted with *B. anthracis* spores, *B. suis*, *F. tularensis*, and *Y. pestis* on aluminum, keyboard, carpet, and joint tape.

B. anthracis spores were not recovered from aluminum, keyboard, or joint tape following a 75-min exposure to HP (fumigate 50 mL with an injection time of 20 min for a nominal concentration of 500 ppmv HP and a peak concentration of 528 ppmv) at an initial RH of 45%, but *B. anthracis* spores were recovered from carpet following a 192-min exposure to HP (three fumigate cycles totaling 50 mL with dwell times between the 15 min injections of approximately 45 min).

B. suis was generally recovered from aluminum, keyboard, carpet, and joint tape following exposures to HP (fumigate 15 mL with an injection time of 15 min) at initial RH conditions of 40% - 50% and 60% - 70%. Two exceptions with no *B. suis* recovery occurred following a 30-min exposure to HP (actual peak concentration of 414 ppmv HP) at an initial RH of 45% on keyboard and a 60-min exposure to HP (actual peak concentration of 303 ppmv HP) on joint tape at an initial RH of 65%.

F. tularensis was exposed to BIOQUELL Clarus S HP (fumigate 15 mL with an injection time of 15 min) at initial RH conditions of 45% and 65%. *F. tularensis* was not recovered from any of the materials tested (aluminum, keyboard, carpet, or joint tape) after 30-min exposures to HP (442 ppmv peak concentration for all materials) at an initial 45% RH or 30-min exposures to HP at an initial 65% RH.

Y. pestis was generally recovered from aluminum, keyboard, carpet, and joint tape following exposures to HP (fumigate 15 mL with an injection time of 15 min) under initial RH conditions of 45% and 65%. Two exceptions with no *Y. pestis* recovery occurred following a 30-min exposure to HP (392 ppmv peak concentration) at an initial 45% RH on aluminum and a 60-min exposure to HP (379 ppmv peak concentration) on joint tape at an initial 65% RH.

STERIS VHP® HP

The STERIS VHP® HP fumigation was conducted at 500 ppmv or 200-250 ppmv HP for various contact times and biological agent/material combinations. All biological agents (*B. anthracis* spores, *B. suis*, *F. tularensis*, vaccinia virus, and *Y. pestis*) were tested on aluminum and keyboard. *B. anthracis* spores, *B. suis* and vaccinia virus were also tested on carpet and joint tape, and *B. anthracis* spores were also tested on laminate, ductwork, concrete, wood, glass, and ceiling tile.

Following a 4-hr exposure to the 500 ppmv HP fumigation cycle, *B. anthracis* spores were not recovered from any of the materials tested (i.e., aluminum, keyboard, carpet, joint tape, laminate, ductwork, concrete, and wood).

With a STERIS VHP® 500 ppmv HP fumigation cycle, *B. suis* was not recovered from carpet or joint tape following a 60-min exposure. *B. suis* was not recovered from aluminum or keyboard following a 90-min exposure.

F. tularensis was not recovered from aluminum or keyboard after a 90-min exposure to the 200-250 ppmv HP fumigation cycle or after a 30-min exposure to the 500 ppmv HP fumigation cycle.

Vaccinia virus was not recovered from carpet or joint tape following a 30-min exposure to the 200-250 ppmv HP fumigation cycle, but vaccinia virus was recovered from aluminum and keyboard. Vaccinia virus was not recovered from keyboard following a 60-min exposure to 200-250 ppmv HP fumigation cycle or a 60-min exposure to the 500 ppmv HP fumigation cycle. Viable vaccinia virus was recovered from aluminum even after a 60-min exposure to the 500 ppmv HP fumigation cycle.

Y. pestis was not recovered from any material tested (aluminum and keyboard) following a 90-min exposure to the 200-250 ppmv HP fumigation cycle.

Fumigation Summary

All fumigation technologies exhibited efficacy against each biological agent with the level of efficacy being dependent on decontamination parameters, e.g., concentration and time, and the type of material inoculated with the biological agent. Based on incidents requiring *B. anthracis* spore decontamination, the real-world criterion for “adequate decontamination” tends to be that no viable spores are recovered after extensive sampling. With this “adequate decontamination” goal in mind, Table ES-2 provides the minimum treatment condition for each fumigation technology that resulted in no viable biological agent being recovered from any of the materials tested. Under the conditions tested, each biological agent was completely rendered non-recoverable by at least one of the technologies, and each of the tested technologies was found to render at least one biological agent non-recoverable.

IMPORTANT NOTE: The results in Table ES-2 are derived from the specific tests, materials, methods of biological agent preparation and application, and conditions that were used in this investigation. The results in Table ES-2 show the decontamination conditions that were identified in which no viable biological agent was recovered. These results should not be interpreted as a comparison of decontamination technologies; concentrations, contact times, or environmental conditions different from those investigated may yield different efficacy results for the various decontamination technologies. Further, demonstration that no viable biological agent was recovered should not be generalized to other materials, environmental conditions, or other methods of application/dispersion of the biological agents.

Table ES-2. Overview of Fumigation Conditions Yielding No Viable Biological Agent Recoveries on Any Tested Material

Biological Agent	Conditions Yielding No Biological Agent Recovery			
	Sabre ClO_2	BIOQUELL Clarus C HP	BIOQUELL Clarus S HP	STERIS VHP® HP
B. anthracis spores	NA	NA	NA	500 ppmv fumigation cycle, 240 min
B. suis	NA	Fumigate 10 min at 8 g/min; dwell at 0.8 g/min; 180-min contact time	NA	500 ppmv fumigation cycle, 90 min
F. tularensis	50-100 ppmv ClO_2, 23 °C, 75% RH, 120 min*	Not Tested	Fumigate 15 mL, initial RH 45% or 65%, 15- or 30-min contact time*	200-250 ppmv fumigation cycle, 90 min or 500 ppmv fumigation cycle, 30 min
Vaccinia Virus	NA	Fumigate 10 min at 8 g/min; dwell at 0.8 g/min; 180-min contact time	Not Tested	NA
Y. pestis	50-100 ppmv ClO_2, 23 ºC, 40% RH or 75% RH, 30 min*	Fumigate 10 min at 8 g/min; dwell at 0.8 g/min; 180-min contact time	NA	200-250 ppmv fumigation cycle, 90 min or 500 ppmv fumigation cycle, 30 min

* Low/no biological agent recovered from the associated positive control confounding the interpretation of the fumigant efficacy data (the lack of biological agent recovery could be attributable to natural degradation rather than fumigant efficacy).

NA = Not applicable, no fumigation test conditions used resulted in no viable recovery on all tested materials.

Biological indicators were used in parallel with the biological agent decontamination testing. The organisms used as the biological indicators were *B. atrophaeus* spores (nominally 10^6 spores) on steel in Tyvek® packaging (Apex Laboratories, Apex, NC), for the Sabre ClO_2 fumigation testing and *G. stearothermophilus* (nominally 1 x 10^6 spores) on stainless steel in Tyvek® packaging for the three HP technologies. The results from qualitative evaluation of the biological indicators did not correlate consistently with the results from quantitative evaluation of viable biological agent remaining on coupons of various materials after decontamination. For example, the *B. atrophaeus* biological indicators used for the Sabre ClO_2 fumigation were all positive for growth after exposure to 3,000 ppmv ClO_2 for the 180-min contact time (the longest time tested), indicative of incomplete kills. The evaluation of decontamination of biological indicators (on steel) is consistent with decontamination of *B. anthracis* on aluminum where viable spores were recovered under all treatment conditions. However, no *B. anthracis* spores were recovered from keyboard or carpet under the same conditions. In contrast, the *G. stearothermophilus* biological indicators were negative for growth (indicating complete kill) after HP fumigation treatments in which viable *B. anthracis*, *B. suis*, and vaccinia virus were recovered from some of the materials tested. For these hardy biological agents, observation of no growth of *G. stearothermophilus* biological indicators cannot be assumed to correlate to no viable biological agent remaining on any material.

Contents

Figures

Tables

Abbreviations and Acronyms

BSC III	Class III biological safety cabinet
°C	degrees Celsius
$CaSO_4$	calcium sulfate
CFU(s)	colony-forming unit(s)
CI	confidence interval
ClO_2	chlorine dioxide
cm	centimeter
$CoCl_2$	cobalt chloride
CT	contact time = concentration x time
CV	coefficient of variation
EPA	U.S. Environmental Protection Agency
g	gram
g	gravity
HEPA	high-efficiency particulate air
HP	hydrogen peroxide
hr	hour
K_2CO_3	potassium carbonate
L	liter
min	minute
mL	milliliter
mm	millimeter
mM	millimolar
NHSRC	National Homeland Security Research Center
NIST	National Institute of Standards and Technology
nm	nanometer
O.D.	optical density
PBS	phosphate buffered saline
PFU(s)	plaque-forming unit(s)
ppm	parts per million
ppmv	parts per million by volume
QA	quality assurance
QC	quality control
QMP	quality management plan
RH	relative humidity
rpm	revolutions per minute
STS	sodium thiosulfate
TSA	technical systems audit
TTEP	Technology Testing and Evaluation Program
µL	microliter

1.0 Introduction

The U.S. Environmental Protection Agency's (EPA's) National Homeland Security Research Center (NHSRC) is helping to protect human health and the environment from adverse impacts resulting from acts of terror. The emphasis of NHSRC is directed toward decontamination and consequence management, water infrastructure protection, and threat and consequence assessment. NHSRC is working to develop tools and information that will help detect the intentional introduction of chemical, biological, or radiological contaminants into buildings or water systems, contain these contaminants, decontaminate buildings or water systems, and dispose of materials resulting from cleanups.

NHSRC's researchers work in partnership with recognized testing organizations; with stakeholder groups consisting of buyers, vendor organizations, and permitters; and with the full participation of individual technology developers in carrying out performance tests on homeland security technologies. NHSRC evaluates the performance of homeland security technologies by developing test plans that are responsive to the needs of stakeholders, conducting tests, collecting and analyzing data, and preparing peer-reviewed reports. All evaluations are conducted in accordance with rigorous quality assurance (QA) protocols to ensure that data of known and high quality are generated and that the results are defensible. Such evaluations provide high-quality information that is useful to decision makers in purchasing or applying the tested technologies. Potential users are provided with unbiased, third-party information that can supplement vendor-provided information. Stakeholder involvement ensures that user needs and perspectives are incorporated into the test design so that useful performance information is produced for each of the tested technologies.

In the interest of expanding our national readiness against highly-ranked threat scenarios, the NHSRC is conducting tests to evaluate the performance of products, methods, and equipment for decontaminating contaminated materials. NHSRC is also investigating the fate (i.e., persistence) of biological, chemical, and radiological agents in the absence of decontamination. For biological agents, persistence reflects the extent that viability or pathogenicity is retained over a defined period of time. Some biological agents are unstable and lose viability or pathogenicity within minutes (min) of their release, thereby diminishing the risk to human health and the environment and the need for decontamination; other agents can remain viable or pathogenic for weeks, months, or years. The persistence of biological agents is influenced by environmental conditions and the materials with which they are in contact. The generation of scientifically defensible persistence data is useful for the proper planning of decontamination efficacy tests and helps formulate first response plans in preparation for possible natural occurrences or intentional releases of biological agents.

The investigation described in this report included three elements:

- Persistence: investigating the change in the % recovery of biological agents over time from various types of indoor building materials under controlled environmental conditions;
- Decontamination efficacy: investigating the log reduction in biological agent extracted from building materials following an experimental fumigation treatment compared to mean log reduction of biological agent extracted from coupons in the absence of the treatment (control); and
- Fumigant damage: observation of visual damage to the surface of building materials caused by the fumigation treatment.

For this report, the persistence of the biological threat agents *Brucella suis*, *Francisella tularensis*, vaccinia virus (a surrogate for the variola virus), and *Yersinia pestis* spiked onto various materials (i.e., aluminum, computer keyboard keys, carpet, and painted joint tape paper) was investigated. Persistence was quantified as the amount of biological agent recovered from the materials following exposure to ambient environmental conditions for up to 7 days. The experimental design allowed us to test whether or not there was significant loss of biological agent over time from the various materials.

This report also summarizes an evaluation of four fumigation technologies: Sabre chlorine dioxide (ClO_2), BIOQUELL Clarus C hydrogen peroxide [HP], BIOQUELL Clarus S HP, and STERIS VHP® HP) that were evaluated with regard to their ability to decontaminate several materials that were spiked with various biological agents including *Bacillus anthracis* spores, *B. suis*, *F. tularensis*, vaccinia virus, and *Y. pestis*.

As used in this investigation, "efficacy" means that the fumigation treatment had the desired effect, at a statistically significant level, of decreasing the amount of viable biological agent recovered from a material, given a fumigant treatment, than from a corresponding untreated positive control. Efficacy is quantified as log reduction.

2.0 Procedures

This section provides an overview of the procedures that were used for the bench-scale investigation of the persistence of biological agents on various materials and the evaluation of fumigation technologies to decontaminate biological agents from indoor surfaces. Testing was performed in accordance with the peer-reviewed and EPA-approved *Test/[Quality Assurance] QA Plan for Systematic Investigation of Fumigant Technologies for Decontamination of Biological Agents from Contaminated Building Materials*[1] and associated amendments excepted as noted in the deviations (Appendix A). The test/QA plan provides additional procedural details that are not included in this report. The general approach and methods, biological agents, and types of materials used are summarized in this section.

2.1 Biological Agents

2.1.1 *Bacillus anthracis*

B. anthracis Ames spores (Battelle culture: USAMRIID M-BAA202) were prepared according to established Battelle Biomedical Research Center procedures.[2] A primary culture of *B. anthracis* Ames from Battelle stock was grown overnight (16-18 hr at 37 °C) in nutrient broth (BD Diagnostic Systems, Sparks, MD) on an orbital shaker (Model 3827, Lab-Line Instruments, Thermo Scientific, Pittsburg, PA) set at 150-200 rpm. An aliquot was used as an inoculum for a scale-up culture that was grown in nutrient broth for 6-8 hr at 150-200 rpm on the orbital shaker. Leighton-Doi Broth (BD Diagnostic Systems, Sparks, MD) inside a BioFlo fermentor (New Brunswick Scientific Co., Inc., Edison, NJ) was inoculated with the scale-up culture and left to grow for approximately 24 hr at 37 ºC. Cultures exhibiting >80% refractile spores were centrifuged (fixed angle rotor) (Avanti J-26 XPI, Beckman Coulter, Brea, CA) at approximately 10,000 – 12,000 x *g* for 15-20 min at 2 ºC-8 ºC. The resultant pellet was washed twice, re-suspended in ice-cold sterile water, heat-shocked (incubated at 60 ºC for 45-60 min), centrifuged, and washed at least twice to remove cellular debris. The spore preparation was purified by centrifuging through a gradient of ice-cold, sterile 58% Hypaque-76 (Nycomed Amersham, Princeton, NJ) at 9,000 x *g* for 2 hr at 2 ºC-8 ºC. The resultant pellet was washed and re-suspended in ice-cold, sterile water and evaluated by phase-contrast microscopy (LEICA CME, Leica Microsystems, Bannockburn, IL). Preparations containing >95% refractile spores with <5% cellular debris were enumerated, diluted with sterile water to approximately 1.0×10^9 colony-forming units (CFUs)/mL and stored at 2 ºC-8 ºC. Details of the method are published in the Journal of Applied Microbiology.[3]

2.1.2 *Brucella suis*

B. suis biotype I (Battelle culture: BRU163) stock solutions were prepared fresh in advance of each day that coupons were spiked. Stock solutions were prepared by transferring *B. suis* colonies from a streak plate (freshly growing or stored at 2 °C – 8 °C for <2 weeks) into 10 mL of brain heart infusion broth (B11059, Fisher Scientific, Pittsburg, PA) that was then incubated at 37 ºC ± 2 ºC with shaking until an increase in turbidity was observed (typically 3 days). The broth culture was added to 40 mL of fresh brain heart infusion broth and incubated overnight to achieve a suitable culture density ($\geq 1 \times 10^8$ CFUs/mL). Alternatively, stock solutions were prepared by transferring *B. suis* colonies from a streak plate (freshly growing or stored at 2 °C- 8 ºC for <2 weeks) into brain heart infusion broth (~15 mL) that was then incubated at 37 ºC ± 2 ºC with shaking until an increase in turbidity was observed.

2.1.3 *Francisella tularensis*

A stock solution of *F. tularensis* LVS (Battelle culture: OSU FTL361) was prepared fresh in advance of each day that the coupons were spiked. Stock solutions were prepared by transferring *F. tularensis* colonies from a streak plate (freshly growing or stored at 2 °C – 8 °C for <2 weeks) into 10 mL of Muller-Hinton broth (OXCM0405B, Fisher Scientific, Pittsburg, PA) (cation adjusted plus IsoVitaleX™ [BD, Franklin Lakes, NJ]) that was then incubated at 37 °C ± 2 °C with shaking until an increase in turbidity was observed. The broth culture was added to 40 mL of fresh Muller-Hinton broth (cation adjusted plus IsoVitaleX™) and the incubation continued for the time necessary to achieve a suitable density ($\geq 1 \times 10^8$ CFUs/mL). Alternatively, stock solutions were prepared by transferring *F. tularensis* colonies from a streak plate (freshly growing or stored <2 weeks) into Muller-Hinton broth (cation adjusted plus IsoVitaleX™) (~15 mL) and incubated at 37 °C ± 2 °C with shaking until an increase in turbidity was observed.

2.1.4 Vaccinia Virus

Stock samples of vaccinia virus, WR (Mouse Neurotropic) (New York City Department of Health, strain (VR119, ATCC, Manassas, VA)) were propagated in Vero cell monolayers (prepared by Battelle). Vero cells were grown in tissue culture flasks at 37 °C ± 2 °C in a 5% CO_2 atmosphere in Eagle minimum essential medium (SH30265.01, Fisher Scientific, Pittsburg, PA) supplemented with 5%-10% fetal bovine serum (30-2020, American Type Culture Collection, Manassas, VA). (Vero cells are typically passaged 20-25 times. A new stock culture is then used.) Confluent Vero cell monolayers were spiked with 1.0 mL of approximately 1 x 10^5 plaque-forming units (PFUs)/mL vaccinia virus. The plates were rocked every 15 min during the 60-min adsorption process. Following adsorption, the Vero monolayers were overlaid with 0.8% methyl cellulose (M0512, Sigma-Aldrich, St. Louis, MO) containing fetal bovine serum, antibiotics (penicillin and streptomycin [30-002-CI, Cellgro, Manassas, VA]) non-essential amino acids (M7145, Sigma-Aldrich, St. Louis, MO) and L-glutamine (25030, Gibco, Carlsbad, CA). The target seeding density was 4 x 10^5 cells/well of a 12-well tissue culture plate (353225, BD, Franklin Lakes, NJ). Following 2-6 days in culture, the Vero cells were harvested and processed through 2-3 freeze-thaw cycles to liberate the viral particles. Cell lysates were centrifuged at approximately 800 x g for 10 min at 4 °C ± 1 °C to remove cell debris. The resultant supernatants were separated into aliquots and frozen at -70 °C until used.

2.1.5 Yersinia pestis

A stock solution of *Y. pestis* CO-92 (Battelle culture: M-YPO166) was prepared fresh in advance of each day that the coupons were spiked. Stock solutions were prepared by transferring *Y. pestis* colonies from a streak plate (freshly growing or stored 2 °C- 8 °C for <2 weeks) into 10 mL of Trypticase soy broth (Remel Inc., Lenexa, Kansas, or Becton Dickinson and Company, Franklin Lakes, NJ) and incubated at 27 °C ± 2 °C with shaking until an increase in turbidity was observed indicative of bacterial replication. The broth culture was added to 40 mL of fresh Trypticase soy broth and incubated until a suitable cell density (≥1 x 10^8 CFUs/mL) was achieved. Alternatively, stock solutions were prepared by transferring *Y. pestis* colonies from a streak plate (freshly growing or stored <2 weeks) into Trypticase soy broth and incubated at 27 °C ± 2 °C with shaking until an increase in turbidity was observed.

Table 2-1. Test Materials

Material	Lot, Batch, or Observation	Manufacturer or Supplier Name	Coupon Size, Width x Length	Material Preparation
Aluminum* (finished)	Aluminum alloy 2024, 1.6 mm thick	Adept Products, Inc., West Jefferson, OH, USA	1.9 cm x 7.5 cm	Autoclave
Keyboard* (computer keys)	Medium grey IBM® shell blanks, acrylonitrile, butadiene, and styrene plastic	DataCal, Gilbert, AZ, USA	1.3 cm x 1.3 cm	Gamma irradiation
Carpet* (industrial)	Style M 7978, color #910	Carpet Corporation of America, Rome, GA, USA	1.9 cm x 7.5 cm	Gamma irradiation
Joint tape* (painted joint tape paper)	SHEETROCK® joint tape (paper tape without glue), roller painted on one side using Martin-Senour paints, one primer (#31-1185) and two finish (flat white, #22-1101) coats	United States Gypsum Company Chicago, IL, USA	1.9 cm x 7.5 cm	Gamma irradiation
Laminate† (decorative)	Grade 10 (nominal thickness 1.2 mm), matte white finish	Solid Surface Design Columbus, OH, USA	1.9 cm x 7.5 cm	Gamma irradiation
Ductwork† (galvanized metal)	Industrial heating, ventilation, and air conditioning standard 24 gauge galvanized steel	Accurate Fabrication Columbus, OH, USA	1.9 cm x 7.5 cm	Autoclave
Concrete† (painted concrete block)	American Society for Testing and Materials International C90 cinder block; brush and roller painted all sides, one coat Martin-Senour latex primer (#71-1185) and one coat Porter® paint latex semi-gloss finish (#919)	Wellnitz Columbus, OH, USA	1.9 cm x 7.5 cm	Autoclave
Wood† (unfinished and untreated pine)	Generic modeling	West Jefferson Hardware West Jefferson, OH, USA	1.9 cm x 7.5 cm	Gamma irradiation
Glass†	American Society for Testing and Materials International C1036	Brooks Brothers Glass and Mirror Service Columbus, OH, USA	1.9 cm x 7.5 cm	Autoclave
Ceiling tile†	Armstrong 954, classic fine textured	Armstrong Columbus, OH, USA	1.9 cm x 7.5 cm	Gamma irradiation

* Material used for persistence and decontamination testing.
† Material used only for decontamination testing.

2.2 Test Materials

Building materials used for persistence testing and/or fumigation experiments are described in Table 2-1. Most of the testing was conducted with aluminum, computer keyboard keys (keyboard), carpet, and painted joint tape paper (joint tape). Test coupons of the materials were cut to the sizes indicated in Table 2-1 from larger pieces of stock material. Coupons were sterilized by autoclaving or gamma irradiation. The selected approach, as shown in Table 2-1, was based on cost-effectiveness and minimization of physical alterations of the material. Autoclaving was performed at Battelle according to an internal standard operating procedure[4], and gamma-irradiation at 40 kilogray was conducted by STERIS Isomedix Services (Libertyville, IL). Gamma-irradiated coupons were sealed in 6 mL Uline poly tubing (Uline, Chicago, IL) to preserve sterility until the coupons were ready for use. Test coupons were each visually inspected prior to being used in any experiment or test. Coupons with anomalies on the application surface were discarded and not used.

2.3 Spiking Coupons

The titer of the *B. anthracis* spores and the vaccinia virus stock suspensions was determined prior to use as described below. For the non-spore forming bacterial species (*Y. pestis, F. tularensis*, and *B. suis*), the stock broth cultures were incubated with shaking until an increase in turbidity was observed indicating bacterial replication. The growth curve was determined by periodically taking samples and measuring the absorbance at 600 nm, i.e., optical density (O.D. 600 nm), using a spectrophotometer (SPECTRAmax Plus 384, Molecular Devices, Sunnyvale, CA) and/or the turbidity (McFarland unit) using the cuvette reader on the spectrophotometer. The CFUs/mL of the sample were simultaneously determined by serial dilution and culture on solid media. A correlation between O.D. 600 nm and/or McFarland units and $\log_{10}$ CFUs/mL was determined by linear regression analysis. The linear equation was used to estimate the CFUs/mL of the growing cultures. The culture suspension was then diluted so that the stock suspension was at the specified titer. The CFUs/mL were determined for the stock suspension at the time of use.

Test and positive control coupons were placed flat and spiked with approximately 1 x 10^7 viable organisms (*B. anthracis* spores, *B. suis*, *F. tularensis*, or *Y. pestis*) per coupon. A 100 µL aliquot of a stock suspension of approximately 1 x 10^8 CFUs/mL of *B. anthracis* spores, *B. suis*, *F. tularensis*, or *Y. pestis* was generally dispensed using a multichannel micropipette (L12-200, Rainin, Oakland, CA) applied as five 10 µL droplets in each of two rows across the surface of the coupon (see Figure 2-1). Only one type of organism was inoculated onto a given test or control coupon. For vaccinia virus, coupons were spiked with approximately 1 x 10^8 PFUs per coupon; a 100 µL aliquot of a stock suspension (approximately 1 x 10^9 PFUs/mL) of vaccinia virus was dispensed using a multichannel micropipette. The solution was applied as five 10 µL droplets in each of two rows across the surface of the coupon.

Because of their small size, the keyboard keys were spiked with a single 100 µL droplet, rather than the 10 x 10 µL droplets used with all of the other materials.

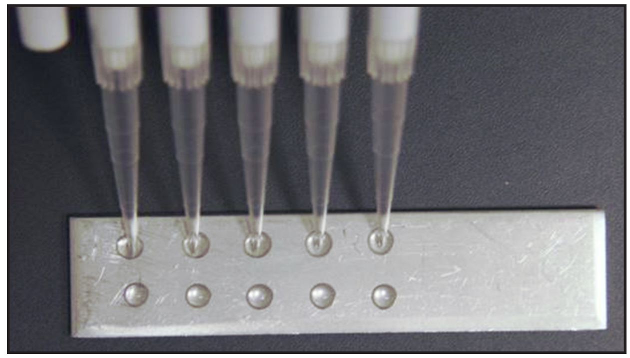


Figure 2-1. Spiking Coupon Using a Multichannel Pipette.

2.4 Test and Control Chambers

2.4.1 Persistence Test Chambers

Bacterial persistence experiments were conducted in sealable chambers (Chefmate® Covered Cake Pan purchased from Target, Minneapolis, MN) with the following dimensions: 34 cm (length) x 24 cm (width) x 8 cm (height). The volume of each chamber was approximately 6.5 L. A maximum of two materials inoculated with one biological agent were placed into a chamber for a given persistence experiment. The chamber used for virus persistence testing is described below (Section 2.4.2).

2.4.2 Decontamination Test and Control Chambers

During testing of the Sabre and BIOQUELL Clarus S technologies, a Compact Glove Box Model 830-ABC (Plas Labs, Inc., Lansing, MI; Figure 2.2), with a total volume of 317 L was used to expose test coupons to the fumigants (decontamination test chamber). For the decontamination evaluations of the STERIS VHP® and BIOQUELL Clarus C technologies, a Class III biological safety cabinet (BSC III) (SG603, Baker, Sanford, ME) was used as the decontamination test chamber; this chamber had a total volume of roughly 1275 L.

The chambers used for positive controls during decontamination testing were generally comparable to the test chambers having the same temperature and RH present in the test chambers at the start of the fumigation cycle. The control chambers were not exposed to fumigants. For the Sabre and STERIS VHP® evaluation, positive controls were placed in sealed vials inside the decontamination chamber.

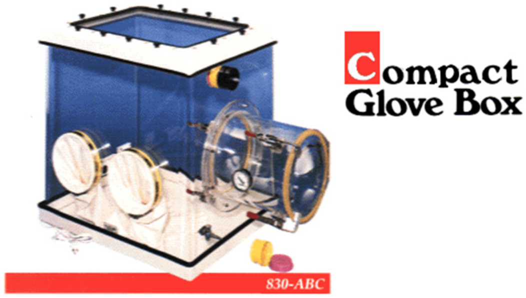


Figure 2-2. Glove Box for Decontamination Testing.

2.5 Monitoring and Controlling Temperature and Relative Humidity

2.5.1 Persistence Test Environmental Monitoring and Control

For bacterial persistence testing, each chamber used contained an open 9 cm Petri dish (08-757-11YZ, Fisher Scientific, Pittsburg, PA) containing a predetermined quantity of the desiccant Indicating Drierite® (98% $CaSO_4$, 2% $CoCl_2$, W A Hammond Co., Xenia, OH) and an open 9 cm Petri dish containing saturated potassium carbonate (K_2CO_3) (Sigma-Aldrich, St. Louis, MO) to maintain the humidity at 35%-45% RH at 22 ºC. The Drierite® desiccant was removed from the chamber after the humidity returned to the lower end of the target range specified in the test/QA plan.[1] The individual chambers were placed in a low temperature incubator (Model LR1201, Thermo Scientific, Pittsburg, PA) capable of maintaining the temperature within the specified range. The temperature and % RH within the chambers were monitored using remote sensors (Model 14-648-53, Fisher Scientific, Pittsburg, PA) that transmitted the data back to a main data logging unit via radio frequency.

For vaccinia virus persistence testing, humidity in the chamber was raised by pulling air from the chamber into a fogging chamber (Battelle, custom manufactured). In the fogging chamber, the air was humidified to ~100% RH using an ultrasonic fog generator (Battelle, custom manufactured). The high humidity air passed out of the fogging chamber through a water trap to remove any liquid water and returned to the chamber. Mixing fans in the chamber caused the humidity to rapidly equilibrate at a higher RH. Drierite® desiccant was placed into the test chamber to lower RH. The temperature and % RH within the chambers were monitored using remote sensors that transmitted the data back to a main data logging unit via radio frequency.

2.5.2 Decontamination Test Environmental Monitoring and Control

The temperature and RH in the test chamber immediately prior to fumigation were at the levels specified for the given trial (see Section 5.0 for technology-specific details). To raise the humidity in the test and control chambers, air from the test or control chamber was pulled into a fogging chamber through an inlet. In the fogging chamber, the air was humidified to ~100% RH using an ultrasonic fog generator. The high humidity air passed out of the fogging chamber through a water trap to remove any liquid water and returned to the test or control chamber. Mixing fans in the test or control chambers caused the humidity to rapidly equilibrate at a higher RH. Drierite® desiccant was placed into the test chamber to lower RH.

The temperature and RH were measured immediately prior to initiating treatment and approximately every 20 min during the evaluation using a hygrometer/thermometer (Model 14-648-53, Fisher Scientific, Pittsburg, PA). While RH readings were taken during fumigation, the RH levels during treatment may be confounded by moisture introduced by the fumigant. Efforts were made only to control the initial RH levels; RH changes within the chamber during fumigation were considered integral to the decontamination method.

2.6 Extracting and Quantifying Biological Agent

For each biological agent, test, positive control, and blank, coupons were placed into individual sterile 50 mL conical vials to which 10.0 mL of sterile extraction buffer was added. Phosphate buffered saline (PBS) (D8537, Sigma, St. Louis, MO) was the extraction buffer for all of the biological agents except *B. anthracis*. PBS with 0.1% Triton X-100 (Sigma-Aldrich®, St. Louis, MO) was the extraction buffer for *B. anthracis* spores. The vials containing the coupons and extraction buffer were agitated on an orbital shaker for 15 min at approximately 200 revolutions per minute (rpm) at room temperature. The resulting liquid extract was removed and serially diluted (typically 1:10 dilutions) in sterile water (up to 10^{-7}, as necessary), for subsequent quantification of biological agent (i.e., CFUs or PFUs of biological agent recovered as determined by a plating the serial dilutions).

2.6.1 Method for Quantifying B. anthracis

For *B. anthracis*, an aliquot (0.1 mL) of the undiluted extract and each serial dilution were spread plated onto tryptic soy agar plates (Remel Inc., Lenexa, KS, or Becton Dickinson and Company, Franklin Lakes, NJ) in triplicate. The cultures were incubated for 18-24 hr at 37 °C ± 2 °C. Colonies were identified and counted manually; well-isolated colonies of *B. anthracis* are white, 2-5 mm in diameter. As shown in Figure 2.3, the flat or slightly convex colonies are irregularly round with undulating edges and a ground glass appearance. The CFUs/mL in the extracts were determined by multiplying the average number of colonies per plate by the reciprocal of the dilution (typically based on plates having colony counts between 30 and 300). The number of detected viable spores extracted from a coupon was calculated according to Equation 1, below.

Equation 1. Total CFUs/coupon = *[(mean CFU plate count x 1/dilution factor)/plated volume] x (extraction buffer volume)*

Where:

Mean CFU plate count	=	average number of colonies counted in the three replicate plates
Plated volume	=	volume that is applied to each plate. In this case 0.1 mL was applied.
Dilution factor	=	portion of the total extraction buffer used to prepare the dilutions
Extraction buffer volume	=	volume of the extraction buffer used to extract the coupon. In this case, 10 mL was applied.

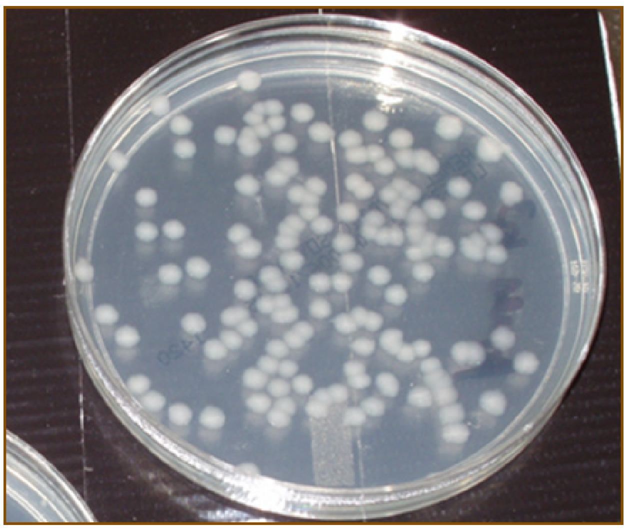


Figure 2-3. *B. anthracis* Ames Colonies on Tryptic Soy Agar.

2.6.2 Method for Quantifying B. suis

For *B. suis*, an aliquot (0.1 mL) of the undiluted extract and each serial dilution were spread-plated onto brain heart infusion agar (Becton Dickinson and Company, Franklin Lakes, NJ) in triplicate. The cultures were incubated for up to 72 hr at 37 °C ± 2 °C. Colonies were identified and counted based on their growth characteristics on the medium. After 48 hr, colonies of *B. suis* are round, 1–2 mm in diameter, convex, pearly-white when viewed from above, with smooth margins.[5] The bacteria recovered from coupons (enumerated as mean CFUs/coupon) were determined in the same manner as described for *B. anthracis* spores in Section 2.6.1.

2.6.3 Method for Quantifying F. tularensis

For *F. tularensis*, an aliquot (0.1 mL) of the undiluted extract and each serial dilution were plated onto chocolate II agar plus IsoVitaleX (Becton Dickinson and Company, Franklin Lakes, NJ) in triplicate. The cultures were incubated for up to 72 hr at 37 °C ± 2 °C. Colonies were identified and counted based on their growth characteristics on the medium; *F. tularensis* colonies after 48 hr are small (1–2 mm in diameter), flat with a shiny surface, white to gray to bluish gray, opaque, with a smooth, entire edge.[6] The bacteria recovered from coupons (enumerated as mean CFUs/coupon) were determined in the same manner as described for *B. anthracis* spores in Section 2.6.1.

2.6.4 Method for Quantifying Vaccinia Virus

For vaccinia virus, an aliquot (0.1 mL) of the undiluted and each appropriate serial dilution of the stock suspension and each coupon extract were plated onto Vero (African green monkey kidney) cell monolayers and allowed to adsorb for 1 hr. Following inoculation and adsorption of virus to the Vero cells, 1.0 mL of 0.7% methylcellulose was added to each well of the 6-well plate. Plates were incubated for 24-48 hr at 37 °C ± 2 °C under 95% air and 5% CO_2. The methylcellulose was removed and 2.0 mL of 0.13% crystal violet (C6158, Sigma-Aldrich, St. Louis, MO) was added and the cells incubated for 30 min. The crystal violet was then removed, the cells were washed with PBS, and the plaques visualized and counted. The virus recovered from the coupons (enumerated as mean PFUs/coupon) was determined by multiplying the average number of PFUs per plate by the reciprocal of the dilution. The PFUs/coupon were calculated by multiplying the PFUs/mL by the volume of the extraction buffer used for each coupon (typically 10 mL per coupon).

2.6.5 Method for Quantifying Y. pestis

For *Y. pestis*, an aliquot (0.1 mL) of the undiluted extract and each serial dilution were plated onto tryptic soy agar (Remel Inc., Lenexa, KS, or Becton Dickinson and Company, Franklin Lakes, NJ) in triplicate. The cultures were incubated for up to 72 hr at 37 °C ± 2 °C. Colonies were identified and counted based on their growth characteristics on the medium. Small grey-white to pale yellow colonies (1–2 mm) are observed at 48-72 hr. Early colonies have a shiny surface described as “hammered copper”. Later colonies have an irregular “fried egg” appearance.[7] The bacteria recovered from coupons (enumerated as mean CFUs/coupon) were determined in the same manner as described for *B. anthracis* spores as described in Section 2.6.1.

2.7 Biological Indicators

Biological indicators were occasionally included in the fumigation testing as specified in the test/QA plan.[1] Specifically:

- For Sabre ClO_2 fumigation *B. atrophaeus* (nominally 1 x 10^6 spores) on steel in Tyvek® packaging (Apex Laboratories, Apex, NC) was used.
- For Clarus S, Clarus C, and STERIS VHP® HP fumigations *Geobacillus stearothermophilus*, nominally 1 x 10^6 spores on stainless steel in Tyvek® packaging, was used.

Biological indicators were used in some preliminary trials to verify that the selected fumigation cycles were efficacious against *G. stearothermophilus.* The presence of viable spores on the biological indicators was determined using a qualitative method. The biological indicators were aseptically transferred into individual tubes containing 30 mL of tryptic soy broth culture medium and capped. The tubes were cultured for 7 days at 37 ºC ± 2 ºC to encourage viable spore germination and subsequent proliferation of vegetative bacteria.

At 1 day and 7 days post-decontamination, the tubes were visually assessed for cloudiness. A cloudy culture medium may indicate "growth" of viable spores. Clear culture medium indicates "no growth" and is consistent with a complete kill of all spores on the biological indicator. Data were presented as "growth" ("+") or "no growth" ("-"). No additional testing (e.g., streak plating) was performed on the biological indicators.

2.8 Calculations and Experimental Design

2.8.1 Percent Recovery

The amount of biological agent that can be recovered may be dependent on the specific material to which it was applied and the type of biological agent. Arithmetic mean % recovery at a given time (*i*) for a given material (*j*) was calculated as:

Equation 2. $$\%\,\text{Recovery}_{ij} = \left(\frac{\overline{C}_{ij}}{\text{Biological Agent Applied}}\right) \times 100$$

Where:

$\overline{C}_{ij}$ is the mean number of viable organisms (CFUs or PFUs) recovered at the i^{th} contact time from the j^{th} material.

The amount of biological agent applied to the material equals the CFUs/mL or PFUs/mL measured in the application control multiplied by the volume (mL) applied to the coupon (i.e., the spike amount). The application control is the enumeration of culturable biological agent in the stock suspension determined by serial dilution, plating, and enumeration.

Statistical analysis (analysis of variance) was used to evaluate whether the mean recovery of the biological agent at a particular contact time on a particular test material was statistically significantly different (p 0.05) from the recovery of biological agent at time zero. Both point estimates and corresponding p-values were determined for each comparison.

2.8.2 Persistence

The persistence testing used a single group time series experimental design, diagrammed as:

$$R \quad O_0 \quad O_1 \quad O_2 \quad O_3 \quad O_4 \quad O_5$$

where time passes from left to right and:

R = Random selection of the test coupons for each time point and type of biological organism.

O_t = Mean measurement (observation) of biological agent extracted from replicate coupons at time

(t) = 0 and five subsequent time periods, designated by subscripts 1-5.

At a given point in time (t), the effect of time on persistence is $O_t – O_0$. The experimental design allowed the following null (H_O) and alternate (H_A) hypotheses to be statistically tested:

$$H_O: O_{(t)} – O_0 = 0$$

$$H_A: O_{(t)} – O_0 < 0$$

That is, the experimental design enables testing of the null or alternate hypothesis that, given an equivalent application of biological agent, the amount of biological agent on the coupons was constant, or, alternatively, decreased over time.

2.8.3 Decontamination Efficacy

Treatments for a given biological agent and building material were defined in terms of the concentration of the fumigant (ClO_2 or HP), temperature, RH, and contact time. To determine the efficacy of the fumigation treatment on a biological agent, a pre-test-post-test control group design was used for each material, biological contaminant, and set of conditions, diagrammed as:

$$\begin{array}{cccc} R & O_1 & X & O_2 \\ R & O_1 & & O_3 \end{array}$$

where time passes from left to right and:

R = Random selection of the test coupons for control, experiment, and type of biological organism.

O = Mean log reduction in measured biological agent extracted from replicate coupons [$O_{1\,(Pretest)}$, $O_{2\,(Treatment)}$, and $O_{3\,(Control)}$], and

X = Experimental variable, in this case the decontamination process.

At a given point in time, the effect of the experimental variable is (O_2 -O_1)-(O_3 -O_1), or simplified, the effect of the experimental variable is $O_{2\,(Treatment)} – O_{3\,(Control)}$. The experimental design was the following null (H_O) and alternate (H_A) hypotheses to be tested statistically:

$$H_O: O_{2\,(Treatment)} - O_{3\,(Control)} = 0$$
$$H_A: O_{2\,(Treatment)} - O_{3\,(Control)} > 0$$

For any particular material, the planned comparisons included decontamination efficacy under given fumigant CT (concentration x contact time), and given environmental conditions (temperature and RH), for a particular biological agent. The experimental design enabled testing of the null hypothesis that there is no difference, or the alternate hypothesis that there is an increase, in the decontamination efficacy using the treatment compared to the control. The design also enabled comparison of rates of removal of biological agents from different material types under specific CT.

Log reduction for a single positive control coupon and a single test coupon is calculated as the difference between the log (recovered biological agent) from the control coupon and the log (recovered biological agent) from the test coupon. At least three complications arise in the use of log reduction to quantify efficacy:

- First, in tests where no viable biological agent is recovered, the log of 0 is mathematically undefined. By convention, a positive number is substituted for the 0. Different substitutions yield different log reduction results, and, when recoveries are low but greater than zero on a coupon, average log reduction values may be higher when viable organisms are recovered than when no viable organisms are recovered.
- Second, there are multiple methods for determining the "average" log reduction when replicate control and test coupons are used. These alternative calculation methods do not yield the same log reduction values or variance.
- Third, some biological agents naturally lose viability over time, at rates that depend on environmental conditions and the material in contact with the biological agent. In determining efficacy, at least two alternative reference points exist: positive controls extracted immediately at time zero ($O_{1\,(Pretest)}$, above), or positive controls ($O_{3\,(Control)}$) inoculated and extracted at the same time as the test coupons ($O_{2\,(Treatment)}$).

If the time zero controls are used as the basis for determining efficacy for a biological agent that loses viability over time, the calculated log reduction value includes both a "natural attenuation" component and the effect of the decontamination technology. The effect of the technology is not differentiated from the natural attenuation. However, if the positive controls have the same history as the test coupons (inoculated at the same time, maintained in the same environmental conditions for the same time), there are controls for "natural attenuation" of the biological agent, but loss of biological agent from the positive control coupons lowers the basis for comparison, resulting in lower log reduction values suggestive of lower efficacy, even if the technology sterilizes the test coupons.

The log reduction complications were addressed in the following manner:

- First, when no viable biological agent is recovered from any coupon in a set, 1 was substituted for the average recovered agent. Substituting a 1 for 0 results in the log reduction being numerically equal to the mean log (recovered biological agent) from the control coupon.
- Second, the log reduction was calculated using two different methods. These calculation methods are described below in this section. The first calculation method was specified in the test/QA plan[1]; the second calculation method is being used in related testing and was included to enable ease of comparisons across studies. The results of using two methods for calculating log reductions are included in this report.
- Third, for biological agents that may lose viability with time, the log reduction was calculated based on positive control coupons ($O_{3\,(Control)}$) with the same history (without treatment) as the test coupons. Thus, the reported log reductions reflect only the incremental contribution of the decontamination treatment to the overall reduction in viable biological agent recovered after a given treatment. Additional information on the combined effect of "natural attenuation" and the treatment technology was provided in the text.

Decontamination efficacy using the method specified in the test/QA plan[1] was calculated as the log reduction in viable biological agent recovered from coupons (enumerated as CFUs or PFUs/coupon) after a given treatment. For a given initial inoculum applied to a coupon, the higher the decontamination efficacy (log reduction) value, the less biological agent remains on the material after a given treatment.

Efficacy was calculated for each individual coupon in a given set of replicates. Efficacy was defined as the extent (by log reduction) to which the biological agent extracted from test coupons after fumigation was less than what was extracted from positive control coupons (not exposed to the fumigant) maintained at the same temperature, RH, and time (test and control coupons were spiked with the same amount and type of biological agent). Efficacy was calculated for *each* test coupon within each combination of contact time (i) and test material (j) according to Equation 3, below.

Equation 3. $$Efficacy_{ij} = \log_{10} \overline{C}_{ij} - \log_{10} N_{ijk}$$

where:

$\overline{C}_{ij}$ = arithmetic mean of the number of viable organisms (enumerated as CFUs or PFUs) recovered from control coupons at the ith contact time and jth test material.

N_{ijk} = number of viable organisms (CFUs or PFUs) recovered on the kth replicate test coupon at the ith contact time and jth test material. If no viable organisms were recovered from N_{ijk}, then N_{ijk} is assigned a value of 1 (because the log of zero is undefined). Therefore, in cases where no viable organisms were recovered from any test coupon:

Equation 4. $$Efficacy_{ij} = \log_{10} \overline{C}_{ij}$$

Mean efficacy (log reduction) and standard deviation were then calculated for the five replicate test coupons of a given material. Statistical analysis (analysis of variance) was used to evaluate whether the efficacy at a particular contact time on a particular test material was significantly different ($p \leq 0.05$) than zero. Both point estimates and corresponding p-values were produced for each comparison.

Decontamination efficacy using the second method was defined as the extent (as log reduction) to which viable *B. anthracis* spores extracted from test coupons after decontamination were less numerous than the viable *B. anthracis* spores extracted from the associated positive control coupons. Mean log reduction is the mean of the base-10 logarithm of recovered agent from the control coupons minus the mean of the base-10 logarithm of recovered agent from the treated coupons. The first steps in this calculation were to determine the logarithm of the CFU count value from each coupon, and then the mean of those logarithm values for each set of positive control and associated test coupons. Efficacy of a decontaminant for a test organism on the ith coupon material was calculated as the difference between those mean log values, i.e.:

Equation 5. $$Efficacy_{ij} = \overline{(\log CFUc_{ij})} - \overline{(\log CFUt_{ij})}$$

Where:

$\log CFUc_{ij}$ = the j individual logarithm values obtained from the positive control coupons.

$\log CFUt_{ij}$ = the j individual logarithm values obtained from the corresponding test coupons.

The overbar designates a mean value.

In tests conducted under this plan, there were five positive controls and five corresponding test coupons (i.e., $j = 5$). In the case where no viable CFUs were found in a coupon extract, a CFU count of 1 was assigned, resulting in a log CFU of zero for that coupon.

The variances (i.e., the square of the standard deviation) of the log $CFUc_{ij}$ and log $CFUt_{ij}$ values were also calculated for both the control and test coupons (i.e., S^2c_{ij} and S^2t_{ij}), and were used to calculate the pooled standard error (SE) for the efficacy value calculated in Equation 6, as follows:

Equation 6. $$SE = \sqrt{\frac{S^2c_{ij}}{5} + \frac{S^2t_{ij}}{5}}$$

where: the number 5 again represents the number j of coupons in both the control and test data sets.

The significance of differences in efficacy across different coupon materials or treatments was assessed based on the 95% confidence interval (CI) of each efficacy result. The 95% CI is:

Equation 7. $$95\%\ CI = Efficacy \pm (1.96 \times SE)$$

Differences in efficacy were judged as significant if the 95% CIs of the two results did not overlap.

A p-value is provided for the probability that the control and treatment recoveries are the same. The p-value is from the two sample t-test with Satterthwaite's method[18, 19] to allow for potentially different variances in the two groups. p-Values less than 0.05 denote less than 1 in 20 chance that a difference as large as or larger than observed would occur by chance if the control and treatment means were truly identical.

In cases where one or more of the treatment coupons had no recovered agent, the mean log reduction of the form ">X" is calculated as the mean of the base-10 logarithm of recovered agent from the control coupons minus the mean of the base-10 logarithm of recovered agent from the treated coupons except that "zero recovery" coupons have a substituted recovered value of "1" (base-10 log is 0). Since the log becomes an increasing negative value below 1 and is undefined at 0, this substitution is necessary and results in a lower bound on the mean log difference, as indicated by the ">". The number of "zero recovery" treatment coupons and the total number of treatment coupons is shown in parentheses. The p-value is from the non-parametric Kolmogorov-Smirnov test.[20] A p-value less than 0.05 denotes less than 1 in 20 chance that results as different as or more different than observed would occur by chance if the distribution of the control and treatment recoveries were truly identical.

2.8.4 CT Calculation

A measure of decontamination efficacy as a function of fumigation treatment is often reported as a CT curve. The product of the fumigant concentration and the contact time of treatment was graphed against the decontamination efficacy. The calculation of the CT value is shown in Equation 8.

Equation 8. $$CT = Concentration \ x \ Contact \ Time$$

The specific units for concentration and contact time depend on the technology in use. For example, for ClO_2 the concentration is in ppmv and the time is in hr. In the investigation of the Clarus C and Clarus S efficacies, the treatments were defined in terms of cycle parameters, rather than a fumigant concentration. Therefore the HP concentration resulting from the specified fumigation cycles is implicit rather than explicit in the CT; the contact time is explicit. Results are reported as log reductions after exposure for a given contact time to a specified fumigation cycle.

2.9 Surface Damage

The physical effect of the decontamination technologies on the materials was also monitored during the evaluation. The qualitative approach provided a gross visual investigation of the damage to the various materials caused by the decontamination technology. Before and after decontamination of the test coupons, the appearance of the decontaminated coupons was visually inspected for any obvious changes in the color, reflectivity, and apparent roughness of the material surfaces. These comparisons were performed for each material, before extraction of the decontaminated test coupons.

3.0
Quality Assurance / Quality Control

Quality assurance/quality control (QC) procedures were performed in accordance with the Quality Management Plan (QMP)[8] and the QA/test plan[1] for the persistence investigation and the technology evaluations. Quality assurance/quality control procedures are summarized below.

3.1 Performance Evaluation Audit

Performance evaluation audits were conducted by the respective laboratory personnel to assess the quality of the results obtained during these experiments. For persistence testing, no performance evaluation audits were performed for biological agents because quantitative standards for these biological materials do not exist. The controls, blanks, and method validation efforts support the biological evaluation results. Table 3-1 summarizes the performance evaluation audits that were performed.

The PE audit for HP concentration compared the output from the Analytical Technology HP gas sensor (B-12 2-Wire Toxic Gas Transmitter, Analytical Technology, Collegeville, PA) to a titration using a Hach HYP-1 test kit. The result of the PE audit met the required tolerance of ± 10% and is shown in Table 3-1. In addition to the PE audit, five additional comparisons of the instrumental reading for HP concentration to the titration method (Hach HYP-1) were performed. In two cases, the comparison exceeded the desired tolerance of ± 10%. These comparisons were not the required PE audit and therefore do not represent a deviation in the test/QA plan, but are provided for information only.

Table 3-1. Performance Evaluation Audits

Measurement	Audit Procedure	Allowable Tolerance	Actual Tolerance
CFU/PFU	Compare to independent count of colonies/plaques	± 10%	0% (100% accurate)
Temperature	Compared to independent thermometer (Model 14-648-53, Fisher Scientific, Pittsburg, PA) value	± 1 °C	<1 °C for 6 of 6 instances
pH (meter)	Measure a standard buffer not used to calibrate the pH meter	± 0.1 pH units	<0.1 pH unit
Flow (mass flow controller; Sierra Instruments)	Compare against mini-BuckTM (National Institute of Standards and Technology [NIST] traceable) primary flow calibrator (mini-Buck M-30, Orlando, FL)	± 5%	0.96%
Time (stopwatch)	Compare against NIST official U.S. time at http://nist.time.gov/timezone.cgi?Eastern/d/-5/java	± 1 sec/min	0 sec/min for 44 of 44 measurements
RH	Compared to independent hygrometer value	± 5%	<5% for 6 of 6 instances
ClO_2 concentration	Titration of standard solution	± 10%	<10%
HP concentration	Hach HYP-1 HP test kit (HYP-1, Hach, Loveland, CO)	± 10%	4.2%
Optical density and wavelength	Compare optical density measurement of the microplate reader (SPECTRAmax Plus 384, Molecular Devices, Sunnyvale, CA)to standard	Optical density ± 1.0%, Wavelength ± 1 nm	10 of 10 readings, all within tolerance
Volume	All micropipettes were certified as calibrated at time of use. Pipettes are recalibrated by gravimetric investigation of pipette performance to manufacturer's specifications every six months by supplier (Rainin Instruments).	± 5%	≤0.5% for 4 of 4 pipettes

3.2 Technical Systems Audit

Battelle QA staff conducted technical systems audits on 6/03/2008 and 10/02/2009 to ensure that the tests were being conducted in accordance with the appropriate QA/test plan[1] and QMP.[8] As part of the audit, test procedures were compared to those specified in the test/QA plan; and data acquisition and handling procedures were reviewed. Observations and findings from the technical systems audit (TSA) were documented and submitted to the Battelle Task Order Leader for response. None of the findings of the TSA required corrective action; only minor issues were noted. TSA records were permanently stored with the Battelle QA Manager.

3.3 Data Quality Audit

At least 10% of the data acquired during the persistence investigation and decontamination technology evaluation were audited by the Battelle QA Manager or a designee. A Battelle QA auditor traced the data from the initial acquisition, through reduction and statistical analysis, to final reporting to ensure the integrity of the reported results. All calculations performed on the data undergoing the audit were checked.

3.4 QA/QC Reporting

Each assessment and audit was documented in accordance with the test/QA plan[1] and QMP.[8] For these tests, no significant findings were noted in any assessment or audit, and no follow-up corrective action was necessary. Copies of the TSA and assessment reports were distributed to the EPA QA Manager and Battelle staff. QA/QC procedures were performed in accordance with the QMP[8] and the test/QA plan[1].

3.5 Deviations from the Test/QA Plan

Two deviations were documented, in compliance with the QMP.[8] *B. anthracis* testing with ClO_2 fumigation, was conducted by having the spiked coupons in closed vials in the test chamber and opening them in sequence so that an appropriate contact time for exposure to ClO_2 was achieved without opening the test chamber which could potentially have resulted in unacceptable variation in the test condition.

The target ranges for application, recovery, and coefficient of variation (CV) of recovery of vegetative bacteria and virus proved very difficult to achieve. Part of the challenge was that the vegetative bacteria may exhibit a rapid loss of viability on one or more of the materials. The materials may have cytotoxic or inactivating properties. Further, unlike spores or virus, vegetative bacteria were actively replicating before (and possibly after) application. These opposing forces, loss of viability and ongoing reproduction, confound efforts to ensure accurate and precise biological agent recoveries at time zero.

Methods used do not allow the bacteria being applied to be precisely determined; therefore, applications of biological agents outside of the target ranges were observed in some cases. The actual inoculation amounts were documented in this report.

Positive control recoveries at time zero were occasionally outside the target recovery range. Further, during persistence testing with *B. suis*, contamination of blanks occurred for a limited number of trials.

There were no methods available to determine more precisely, before the testing, the amount of vegetative bacteria applied, to prevent loss of viable bacteria and virus during drying on certain materials, or to reduce the CV of recoveries from the various materials. Tests exceeding the target ranges and/or CV were noted in Appendix A and, where appropriate, in data tables; the tests were not repeated.

The second deviation documents that trials were not repeated when:

- The amounts of biological agent inoculated onto the coupons were outside of the acceptance criteria (≥10% - ≤120% for spores; 1 log for vegetative organisms).
- Positive control recoveries were outside of the acceptance criteria (≥10% - ≤120% of the spores and vegetative bacteria applied; $\geq 1 \times 10^5$ PFUs/coupon for virus.
- Blank contamination occurred.

The deviations (tests not repeated) are provided in Appendix A. Where applicable, the data impacted by the deviations are noted in appropriate tables. The higher than expected variability in the biological agent applications, positive control recoveries, and the rare contaminated blank coupons were believed to have had a minimal impact on the test results.

4.0 Recovery and Persistence Results

4.1 Recovery Results

The amount of biological agent that can be recovered may be dependent on the specific material to which it is applied and the type of biological agent. For combinations of biological agent and materials where recoveries had not previously been determined by the laboratory, method demonstration was performed to determine the percent recoveries by extraction in PBS. Results of the recovery demonstrations are presented in Table 4-1. The recovery demonstrations included:

- *B. suis* from aluminum, keyboard, carpet, and joint tape.
- *B. anthracis* from aluminum and keyboard.
- *F. tularensis* from aluminum, keyboard, and joint tape.
- Vaccinia virus from aluminum, keyboard, carpet, and joint tape.
- *Y. pestis* from aluminum, keyboard, carpet, and joint tape.

In addition to the PBS extractions, two modifications of the extraction solution were tested in an effort to improve recoveries of *F. tularensis* and *B. suis*. For *B. suis* and *F. tularensis*, the extraction solution was modified by addition of catalase (Roche, Indianapolis, IN) to the PBS (PBS with 0.1% catalase). The hypothesis was that the catalase might serve as an antioxidant to reduce the rate at which *F. tularensis* and *B. suis* viability (and corresponding recovery) declined. The addition of catalase did not appreciably change the recoveries and therefore catalase was not added to the methodology. For *F. tularensis,* an additional extraction solution was tested by amending the PBS with 100 mM trehalose (Fisher Scientific, Pittsburg, PA). The hypothesis was that trehalose might increase the persistence of *F. tularensis* as had been demonstrated for *Escherichia coli* and *Bacillus thuringiensis*.[9] Trehalose, likewise, did not substantially improve recovery and, therefore, trehalose was not added to the methodology. Based on these extraction and recovery results the Task Order Project Officer selected the extraction methods described in Section 2.6.

For undetermined reasons, no *B. suis* was recovered from keyboard keys in the first extraction and recovery from aluminum was also low. However, in subsequent testing *B. suis* was recovered from keyboard keys and aluminum at useful levels (see Table 4-2).

For undetermined reasons, no *F. tularensis* was recovered from joint tape. Attempts to improve recovery by incorporating catalase into the extraction buffer did not yield any viable *F. tularensis*. Therefore, catalase was not added to the extraction buffer for subsequent *F. tularensis* persistence testing.

Table 4-1. Biological Agent Recovery

Agent / Material	**Unique Aspects of Extraction Approach**	**Replicate Coupons**	**Spike Amount**	**Agent Recovered**	
B. anthracis			**CFU/coupon**	**CFU/coupon***	**%***
Aluminum	None, enumerated 7/26/07	3	$9.87 \times 10^{7\dagger}$	$3.79 \pm 0.66 \times 10^{6}$	6.39 ± 0.67
Keyboard	None, enumerated 7/26/07	3	$9.87 \times 10^{7\dagger}$	$4.25 \pm 0.44 \times 10^{6}$	7.18 ± 0.44
Aluminum	None, enumerated 8/1/07	3	$9.17 \times 10^{7\dagger}$	$4.18 \pm 0.07 \times 10^{6}$	7.60 ± 0.07
Keyboard	None, enumerated 8/1/07	3	$9.17 \times 10^{7\dagger}$	$4.61 \pm 0.62 \times 10^{6}$	8.38 ± 0.68
B. suis			**CFU/coupon**	**CFU/coupon***	**%***
Aluminum	None	3	1.71×10^{7}	$1.75 \pm 1.67 \times 10^{5}$	1.70 ± 0.98
Keyboard	None	3	1.71×10^{7}	0.00 ± 0.00	0.00 ± 0.00
Carpet	None	3	1.71×10^{7}	$2.45 \pm 0.52 \times 10^{6}$	23.9 ± 3.02
Joint tape	None	3	1.71×10^{7}	$1.85 \pm 1.92 \times 10^{5}$	1.80 ± 1.12
Aluminum	Catalase, 0 hr	5	4.10×10^{6}	$3.12 \pm 0.22 \times 10^{6}$	76.0 ± 5.47
Joint tape	Catalase, 0 hr	5	4.10×10^{6}	$2.49 \pm 0.53 \times 10^{5}$	6.07 ± 1.29
Aluminum	Catalase, 5 hr	5	4.10×10^{6}	$3.48 \pm 0.58 \times 10^{6}$	84.9 ± 14.2
Joint tape	Catalase, 5 hr	5	4.10×10^{6}	$9.69 \pm 0.88 \times 10^{4}$	2.36 ± 0.21
F. tularensis			**CFU/coupon**	**CFU/coupon***	**%***
Aluminum	None	3	6.80×10^{6}	$2.70 \pm 2.17 \times 10^{5}$	6.62 ± 3.18
Aluminum	0 hr, no trehalose	5	9.40×10^{6}	$7.95 \pm 2.17 \times 10^{6}$	84.6 ± 23.1
Aluminum	0 hr with 100 mM trehalose	5	1.11×10^{7}	$1.25 \pm 0.08 \times 10^{7}$	113 ± 6.95
Aluminum	1 hr, no trehalose	5	9.40×10^{6}	$2.31 \pm 1.43 \times 10^{5}$	2.46 ± 1.52
Aluminum	1 hr with 100 mM trehalose	5	1.11×10^{7}	$8.33 \pm 1.60 \times 10^{5}$	7.48 ± 1.44
Aluminum	10 drops without trehalose	5	NA	$1.68 \pm 0.10 \times 10^{5}$	NA
Aluminum	Single drop without trehalose	5	NA	$2.44 \pm 1.51 \times 10^{4}$	NA
Keyboard	Single drop without trehalose	5	NA	$1.70 \pm 0.24 \times 10^{5}$	NA
Keyboard	Single drop with trehalose	5	NA	$1.69 \pm 0.53 \times 10^{5}$	NA
Aluminum	0.1% Catalase, 0 hr	5	5.37×10^{6}	$1.61 \pm 0.99 \times 10^{4}$	0.30 ± 0.18
Joint tape	0.1% Catalase, 0 hr	5	5.37×10^{6}	0.00 ± 0.00	0.00 ± 0.00
Aluminum	0.1% Catalase, 5 hr	5	5.37×10^{6}	$1.19 \pm 0.70 \times 10^{4}$	0.22 ± 0.13
Joint tape	0.1% Catalase, 5 hr	5	5.37×10^{6}	0.00 ± 0.00	0.00 ± 0.00
Vaccinia Virus			**PFU/coupon**	**PFU/coupon***	**%***
Aluminum	0-min dry time	5	9.11×10^{7}	$6.68 \pm 8.43 \times 10^{6}$	47.0 ± 9.26
Keyboard	0-min dry time	5	6.38×10^{7}	$3.97 \pm 1.97 \times 10^{7}$	62.3 ± 30.8
Carpet	0-min dry time	5	6.38×10^{7}	$3.44 \pm 0.95 \times 10^{7}$	54.0 ± 14.9
Joint tape	0-min dry time	5	9.11×10^{7}	$7.34 \pm 0.97 \times 10^{7}$	80.6 ± 10.6
Aluminum	1 hr dry time	5	9.11×10^{7}	$3.46 \pm 1.44 \times 10^{7}$	38.0 ± 15.8
Keyboard	1 hr dry time	5	6.38×10^{7}	$3.68 \pm 1.51 \times 10^{7}$	57.7 ± 23.7
Carpet	1 hr dry time	5	6.38×10^{7}	$2.87 \pm 0.90 \times 10^{7}$	45.1 ± 14.1
Joint tape	1 hr dry time	5	9.11×10^{7}	$7.03 \pm 3.41 \times 10^{6}$	7.71 ± 3.75
Y. pestis			**CFU/coupon**	**CFU/coupon***	**%***
Aluminum	None	3	4.20×10^{6}	$3.75 \pm 1.24 \times 10^{3}$	0.15 ± 0.03
Keyboard	None	3	4.20×10^{6}	$7.49 \pm 21.6 \times 10^{2}$	0.03 ± 0.05
Carpet	None	3	4.20×10^{6}	$1.61 \pm 1.86 \times 10^{3}$	0.06 ± 0.04
Joint tape	None	3	4.20×10^{6}	$1.93 \pm 1.67 \times 10^{2}$	0.01 ± 0.00

NA = Not available.
* Data are expressed as mean ± standard deviation.
† Application was inadvertently about 1 log higher than the target 1×10^{7} CFUs/coupon.

4.2 Persistence Testing

Persistence results for each material and environmental condition are summarized in Tables 4-2 through 4-5 and Figures 4-1 through 4-4. Persistence testing was not conducted with *B. anthracis* spores, which are known to survive for decades under ambient and adverse conditions.[9, 10, 11]

Except as noted, in Section 4.2.1, no viable organisms were recovered from any blank coupon.

4.2.1 B. suis Persistence

The persistence results obtained for *B. suis* are summarized in Table 4-2 and Figure 4-1. *B. suis* persisted on aluminum, keyboard, and carpet for the longest exposure duration tested (7 days). Low levels of *B. suis* were recovered after 4 hrs, but were not recovered after 8 hr of exposure on joint tape.

Table 4-2. *B. suis* Persistence

Duration	Test Temperature (oC)	Test RH (%)	Spike Amount (CFU/ coupon)	Mean Recovered *B. suis* (CFU/coupon)*	Mean (%) Recovered *B. suis*
Aluminum					
0 hr†	NA	NA	3.30 x 10^7	1.84 ± 0.15 x 10^7 [‡]	56
2 hr	20 - 24	24 - 38	3.30 x 10^7	8.82 ± 4.16 x 10^6 [§]	27
4 hr	20 - 25	25 - 41	3.30 x 10^7	3.69 ± 1.36 x 10^6	11
8 hr	20 - 25	24 - 37	2.72 x 10^7 [#]	1.19 ± 0.20 x 10^7	44
3 days	20 - 25	26 - 42	3.30 x 10^7	8.45 ± 1.47 x 10^5	2.6
7 days	20 - 25	27 - 42	3.30 x 10^7	8.62 ± 3.23 x 10^5	2.6
Keyboard					
0 hr†	NA	NA	2.30 x 10^7	2.45 ± 0.25 x 10^7	106
2 hr	21 - 22	40 - 51	2.30 x 10^7	2.45 ± 0.11 x 10^7	106
4 hr	20 - 22	40 - 51	2.30 x 10^7	2.32 ± 0.26 x 10^7	101
8 hr	20 - 22	35 - 52	2.30 x 10^7	1.39 ± 0.30 x 10^7	60
3 days	18 - 22	35 - 60	2.30 x 10^7	2.96 ± 1.09 x 10^6 [¶]	13
7 days	18 - 22	37 - 60	2.30 x 10^7	1.06 ± 0.06 x 10^6	4.6
Carpet					
0 hr†	NA	NA	3.30 x 10^7	1.76 ± 0.07 x 10^7	53
2 hr	20 - 24	24 - 38	3.30 x 10^7	3.23 ± 3.03 x 10^5	<1
4 hr	20 - 25	25 - 41	3.30 x 10^7	4.91 ± 1.66 x 10^4	<1
8 hr	20 - 25	24 - 37	2.72 x 10^7 [#]	5.00 ± 4.92 x 10^4	<1
3 days	20 - 25	26 - 42	3.30 x 10^7	3.91 ± 2.36 x 10^3	<1
7 days	20 - 25	27 - 42	3.30 x 10^7	4.66 ± 4.35 x 10^2	<1
Joint Tape					
0 hr†	NA	NA	2.30 x 10^7	2.13 ± 0.40 x 10^7	93
2 hr	21 - 22	40 - 51	2.30 x 10^7	1.60 ± 1.90 x 10^2	<1
4 hr	20 - 22	40 - 51	2.30 x 10^7	3.26 ± 2.37 x 10^1	<1
8 hr	20 - 22	35 - 52	2.30 x 10^7	0.00 ± 0.00	0
3 days	18 - 22	35 - 60	2.30 x 10^7	0.00 ± 0.00	0
7 days	18 - 22	37 - 60	2.30 x 10^7	0.00 ± 0.00	0

NA = Not applicable.

[*] Data are expressed as mean ± standard deviation of five replicates.

[†] 0 hr durations are positive control coupons that are spiked and extracted at time zero.

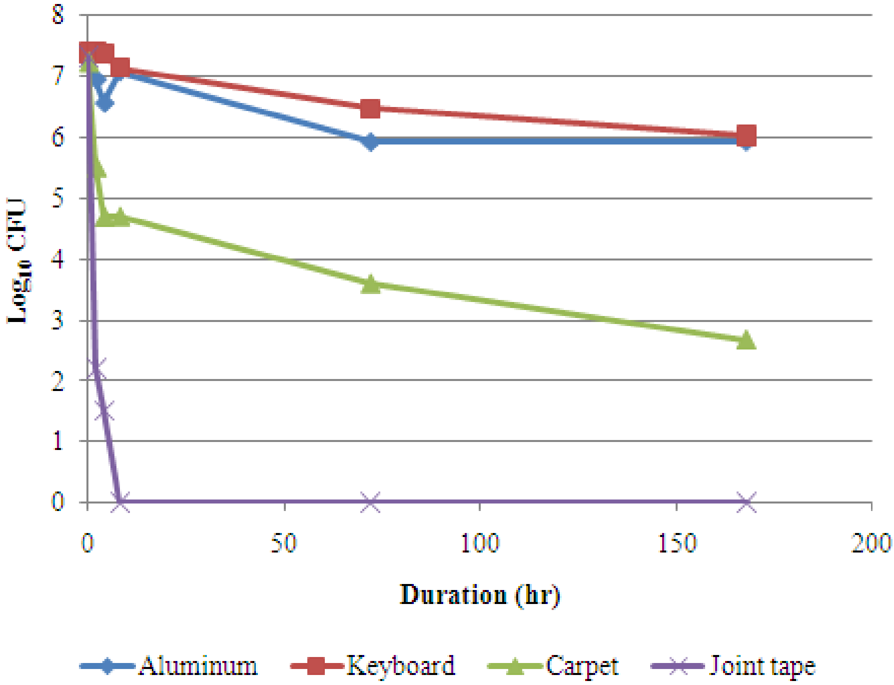
[‡] 2.30 x 10^2 CFUs/coupon were recovered from the associated blank coupon.

[§] 6.70 x 10^1 CFUs/coupon were recovered from the associated blank coupon.

[#] Due to a spill associated with the 8 hr-test with aluminum and carpet, these materials were retested on a different day and therefore have spike amounts different from associated tests.

[¶] 3.00 x 10^1 CFUs/coupon were recovered from the associated blank coupon.

Figure 4-1. *B. suis* Persistence.



4.2.2 *F. tularensis* Persistence

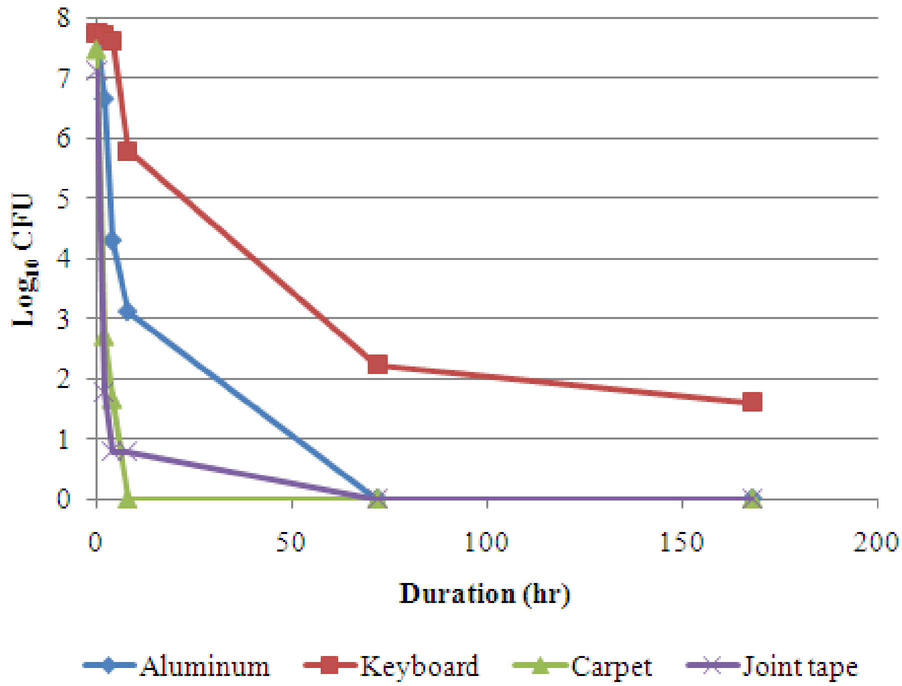
The results obtained for persistence of *F. tularensis* are summarized in Table 4-3 and Figure 4-2. *F. tularensis* persisted 7 days (the longest duration tested) only on keyboard. Persistence of *F. tularensis* on other materials was: aluminum (8 hr), carpet (4 hr), and joint tape (8 hr).

Table 4-3. *F. tularensis* Persistence

Duration	Test Temperature (°C)	Test RH (%)	Spike Amount (CFUs/coupon)	Mean Recovered *F. tularensis* (CFUs/coupon)*	Mean (%) Recovered *F. tularensis*
Aluminum					
0 hr†	NA	NA	4.47×10^{7}	$5.37 \pm 0.75 \times 10^{7}$	120‡
2 hr	NM	NM	4.47×10^{7}	$4.40 \pm 3.55 \times 10^{6}$	9.8
4 hr	NM	NM	4.47×10^{7}	$1.95 \pm 0.81 \times 10^{4}$	<1
8 hr	20 - 24	25 - 45	4.47×10^{7}	$1.29 \pm 1.04 \times 10^{3}$	<1
3 days	22 - 24	21 - 42	4.47×10^{7}	0.00 ± 0.00	0
7 days	22 - 24	22 - 41	4.47×10^{7}	0.00 ± 0.00	0
Keyboard					
0 hr†	NA	NA	4.47×10^{7}	$5.40 \pm 0.83 \times 10^{7}$‡	121
2 hr	NM	NM	4.47×10^{7}	$5.04 \pm 3.02 \times 10^{7}$	113
4 hr	NM	NM	4.47×10^{7}	$4.12 \pm 0.75 \times 10^{7}$	92
8 hr	20 - 24	25 – 45	4.47×10^{7}	$6.05 \pm 4.17 \times 10^{5}$	1.4
3 days	22 - 24	21 - 42	4.47×10^{7}	$1.67 \pm 0.97 \times 10^{2}$	<1
7 days	22 - 24	22 - 41	4.47×10^{7}	$3.98 \pm 3.66 \times 10^{1}$	<1
Carpet					
0 hr†	NA	NA	1.77×10^{7}	$3.00 \pm 1.02 \times 10^{7}$‡	169
2 hr	NM	NM	1.77×10^{7}	$5.07 \pm 3.48 \times 10^{2}$	<1
4 hr	NM	NM	1.77×10^{7}	$4.54 \pm 3.87 \times 10^{1}$	<1
8 hr	20 - 25	22 - 36	1.77×10^{7}	0.00 ± 0.00	0
3 days	22 - 24	23 - 38	1.77×10^{7}	0.00 ± 0.00	0
7 days	22 - 23	24 - 40	1.77×10^{7}	0.00 ± 0.00	0
Joint Tape					
0 hr†	NA	NA	1.77×10^{7}	$1.29 \pm 0.25 \times 10^{7}$	73
2 hr	NM	NM	1.77×10^{7}	$6.00 \pm 6.63 \times 10^{1}$	<1
4 hr	NM	NM	1.77×10^{7}	$6.00 \pm 13.4 \times 10^{0}$	<1
8 hr	20 - 25	22 - 36	1.77×10^{7}	$6.00 \pm 13.4 \times 10^{0}$	<1
3 days	22 - 24	23 - 38	1.77×10^{7}	0.00 ± 0.00	0
7 days	22 - 23	24 - 40	1.77×10^{7}	0.00 ± 0.00	0

NA = Not applicable.
NM = Not monitored.
* Data are expressed as mean ± standard deviation of five replicates.
† 0 hr durations are positive control coupons that are spiked and extracted at time zero.
‡Exceeds target recovery of ≤120% of spike amount.

Figure 4-2. *F. tularensis* Persistence.



4.2.3 Vaccinia Virus Persistence

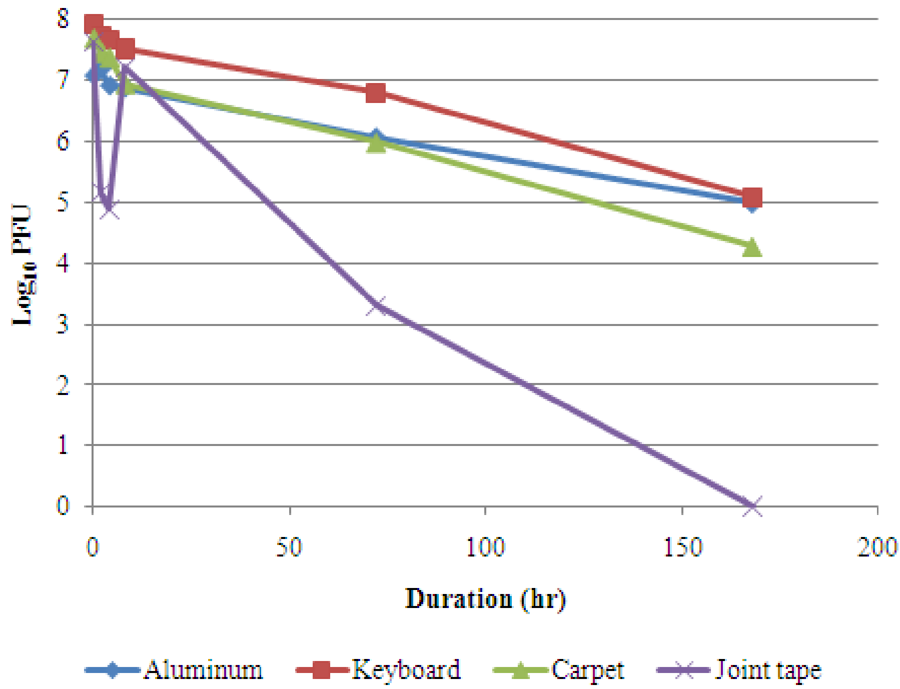
The results obtained for the persistence of vaccinia virus are summarized in Table 4-4 and Figure 4-3. Vaccinia virus persisted 7 days on aluminum, keyboard, and carpet. On joint tape, vaccinia virus was recovered after 3 days but not after the 7-day duration.

Table 4-4. Vaccinia Virus Persistence

Duration	Test Temperature (°C)	Test RH (%)	Spike Amount (PFU/ coupon)	Mean Recovered Vaccinia Virus (PFU/coupon)*	Mean (%) Recovered Vaccinia Virus
Aluminum					
0 hr†	NA	NA	1.14×10^8	$1.19 \pm 1.12 \times 10^7$	47
2 hr	23 - 23	37 - 45	1.14×10^8	$1.57 \pm 1.30 \times 10^7$	57
4 hr	22 - 23	37 - 45	1.14×10^8	$8.15 \pm 6.72 \times 10^6$	37
8 hr	21 - 23	37 - 45	1.14×10^8	$7.53 \pm 17.5 \times 10^6$	24
3 days	21 - 23	37 - 45	1.14×10^8	$1.16 \pm 0.40 \times 10^6$	5.0
7 days	21 - 23	37 - 45	1.14×10^8	$9.70 \pm 7.69 \times 10^4$	<1
Keyboard					
0 hr†	NA	NA	1.14×10^8	$8.34 \pm 1.10 \times 10^7$	73
2 hr	23 - 23	37 - 45	1.14×10^8	$5.13 \pm 2.08 \times 10^7$	45
4 hr	22 - 23	37 - 45	1.14×10^8	$4.60 \pm 2.33 \times 10^7$	40
8 hr	21 - 23	37 - 45	1.14×10^8	$3.33 \pm 0.59 \times 10^7$	29
3 days	21 - 23	37 - 45	1.14×10^8	$6.21 \pm 1.67 \times 10^6$	5.5
7 days	21 - 23	37 - 45	1.14×10^8	$1.19 \pm 0.40 \times 10^5$	<1
Carpet					
0 hr†	NA	NA	1.14×10^8	$5.08 \pm 1.16 \times 10^7$	45
2 hr	23 - 23	37 - 45	1.14×10^8	$2.94 \pm 2.26 \times 10^7$	26
4 hr	22 - 23	37 - 45	1.14×10^8	$2.43 \pm 0.41 \times 10^7$	21
8 hr	21 - 23	37 - 45	1.14×10^8	$8.94 \pm 4.26 \times 10^6$	7.8
3 days	21 - 23	37 - 45	1.14×10^8	$9.66 \pm 2.07 \times 10^5$	<1
7 days	21 - 23	37 - 45	1.14×10^8	$1.87 \pm 1.34 \times 10^4$	<1
Joint Tape					
0 hr†	NA	NA	1.14×10^8	$4.27 \pm 1.09 \times 10^{7‡}$	37
2 hr	23 - 23	37 - 45	1.14×10^8	$1.46 \pm 0.71 \times 10^5$	<1
4 hr	22 - 23	37 - 45	1.14×10^8	$7.69 \pm 3.14 \times 10^4$	<1
8 hr	21 - 23	37 - 45	1.14×10^8	$1.64 \pm 2.28 \times 10^7$	14
3 days	21 - 23	37 - 45	1.14×10^8	$2.01 \pm 2.75 \times 10^3$	<1
7 days	21 - 23	37 - 45	1.14×10^8	0.00 ± 0.00	0

NA = Not applicable.
* Data are expressed as mean ± standard deviation of five replicates.
† 0 hr durations are positive control coupons that are spiked and extracted at time zero.
‡Exceeded the positive control CV target of ≤25%.

Figure 4-3. Vaccinia Virus Persistence.



4.2.4 *Y. pestis* Persistence

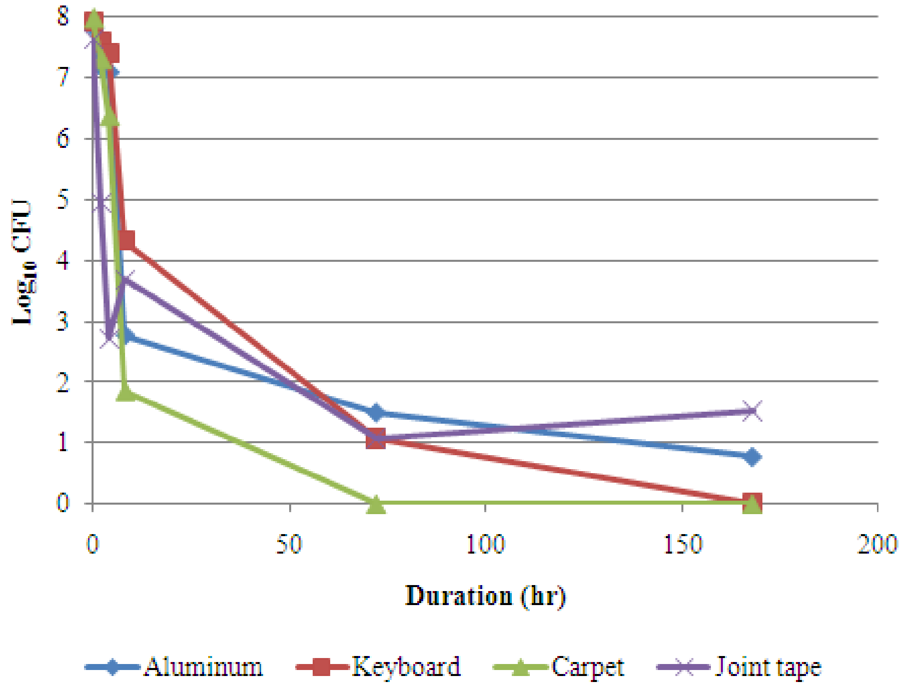
The *Y. pestis* persistence results are summarized in Table 4-5 and Figure 4-4. *Y. pestis* persisted at relatively low levels for 7 days on aluminum and joint tape. *Y. pestis* persisted for 3 days on keyboard and 8 hr on carpet.

Table 4-5. *Y. pestis* Persistence

Duration	**Test Temperature (°C)**	**Test RH (%)**	**Spike Amount (CFUs/ coupon)**	**Mean Recovered *Y. pestis* (CFUs/coupon)***	**Mean (%) Recovered *Y. pestis***
Aluminum					
0 hr†	NA	NA	2.90×10^7	$6.49 \pm 3.06 \times 10^7$	224‡
2 hr	20 - 20	58 - 64	2.90×10^7	$1.45 \pm 0.92 \times 10^7$	50
4 hr	20 - 21	52 - 62	2.90×10^7	$1.23 \pm 0.95 \times 10^7$	42
8 hr	20 - 21	36 - 62	2.90×10^7	$5.92 \pm 7.31 \times 10^2$	<1
3 days	20 - 21	36 - 59	2.90×10^7	$3.20 \pm 4.09 \times 10^1$	<1
7 days	20 - 21	36 - 61	2.90×10^7	$6.00 \pm 13.4 \times 10^0$	<1
Keyboard					
0 hr†	NA	NA	2.86×10^7	$8.23 \pm 1.26 \times 10^7$	288‡
2 hr	20 - 21	49 - 75	2.86×10^7	$3.89 \pm 3.27 \times 10^7$	136‡
4 hr	20 - 21	51 - 71	2.86×10^7	$2.52 \pm 2.39 \times 10^7$	88
8 hr	20 - 21	39 - 71	2.86×10^7	$2.12 \pm 1.49 \times 10^4$	<1
3 days	20 - 21	40 - 70	2.86×10^7	$1.20 \pm 1.64 \times 10^1$	<1
7 days	19 - 20	40 - 73	2.86×10^7	0.00 ± 0.00	0
Carpet					
0 hr†	NA	NA	2.90×10^7	$9.55 \pm 0.84 \times 10^7$	329‡
2 hr	20 - 20	58 - 64	2.90×10^7	$2.02 \pm 0.79 \times 10^7$	70
4 hr	20 - 21	52 - 62	2.90×10^7	$2.34 \pm 5.23 \times 10^6$	8.1
8 hr	20 - 21	36 - 62	2.90×10^7	$7.20 \pm 12.8 \times 10^1$	<1
3 days	20 - 21	36 - 59	2.90×10^7	0.00 ± 0.00	0
7 days	20 - 21	36 - 61	2.90×10^7	0.00 ± 0.00	0
Joint Tape					
0 hr†	NA	NA	2.86×10^7	$4.30 \pm 3.42 \times 10^7$	150‡
2 hr	20 - 21	49 - 75	2.86×10^7	$8.69 \pm 11.4 \times 10^4$	<1
4 hr	20 - 21	51 - 71	2.86×10^7	$5.14 \pm 8.28 \times 10^2$	<1
8 hr	20 - 21	39 - 71	2.86×10^7	$4.81 \pm 7.12 \times 10^3$	<1
3 days	20 - 21	40 - 70	2.86×10^7	$1.20 \pm 1.64 \times 10^1$	<1
7 days	19 - 20	40 - 73	2.86×10^7	$3.34 \pm 3.35 \times 10^1$	<1

NA = Not applicable.
* Data are expressed as mean ± standard deviation of five replicates.
† 0 hr durations are positive control coupons that are spiked and extracted at time zero.
‡Exceeds target recovery of ≤120% of spike amount.

Figure 4-4. *Y. pestis* Persistence.



4.2.5 Summary of Persistence Testing

Table 4-6 provides a summary of persistence data, calculated as the difference in the mean log of viable bacteria (enumerated as CFUs/coupon) or virus (enumerated as PFUs/coupon) recovered from coupons at time zero and the mean log viable spores recovered from coupons at a later specified time. The 95% CI and p-value are also shown. Significant reduction in viable biological agent was observed for most agent/material combinations within 2 hr. Every biological agent exhibited significant loss of viability within 2 hr from at least one material.

Table 4-6. Summary of Persistence Results Calculated as Mean Log Reduction

Agent	Material	Mean Log Reduction (95% confidence interval) and p-Value* or Mean Log Reduction (# of persistence coupons with zero recovery/# of persistence coupons) and p-Value†				
		2 hr	4 hr	8 hr	3 day	7 day
B. suis	Aluminum	**0.37 (0.09, 0.66) p=0.0374**	**0.72 (0.55, 0.90) p=0.0004**	**0.19 (0.11, 0.28) p=0.0030**	**1.34 (1.26, 1.43) p<0.0001**	**1.35 (1.19, 1.51) p<0.0001**
	Carpet	**1.89 (1.47, 2.30) p=0.0005**	**2.57 (2.42, 2.72) p<0.0001**	**2.68 (2.31, 3.05) p<0.0001**	**3.71 (3.47, 3.95) p<0.0001**	**>5.51 (2/5) p=0.0079**
	Keyboard	-0.00 (-0.05, 0.05) p=0.9364	0.02 (-0.04, 0.09) p=0.4478	**0.25 (0.14, 0.36) p=0.0022**	**0.94 (0.75, 1.13) p=0.0002**	**1.36 (1.31, 1.41) p<0.0001**
	Painted Joint Tape	**>5.63 (1/5) p=0.0079**	**>6.05 (1/5) p=0.0079**	**>7.32 (5/5) p=0.0079**	**>7.32 (5/5) p=0.0079**	**>7.32 (5/5) p=0.0079**
F. tularensis	Aluminum	**1.48 (0.48, 2.47) p=0.0265**	**3.47 (3.26, 3.67) p<0.0001**	**4.73 (4.36, 5.10) p<0.0001**	**>7.73 (5/5) p=0.0079**	**>7.73 (5/5) p=0.0079**
	Carpet	**>5.24 (1/5) p=0.0079**	**>6.10 (1/5) p=0.0079**	**>7.46 (5/5) p=0.0079**	**>7.46 (5/5) p=0.0079**	**>7.46 (5/5) p=0.0079**
	Keyboard	0.11 (-0.24, 0.46) p=0.5107	**0.12 (0.01, 0.23) p=0.0414**	**2.09 (1.61, 2.57) p=0.0005**	**5.61 (5.21, 6.00) p<0.0001**	**>6.42 (1/5) p=0.0079**
	Painted Joint Tape	**>5.70 (1/5) p=0.0079**	**>6.81 (4/5) p=0.0079**	**>6.81 (4/5) p=0.0079**	**>7.10 (5/5) p=0.0079**	**>7.10 (5/5) p=0.0079**
Vaccinia Virus	Aluminum	-0.08 (-0.21, 0.05) p=0.1879	0.10 (-0.02, 0.22) p=0.0934	>1.70 (1/5) p=0.0794	**0.97 (0.87, 1.07) p<0.0001**	**2.09 (1.96, 2.22) p<0.0001**
	Carpet	0.32 (-0.02, 0.66) p=0.0827	**0.32 (0.19, 0.45) p=0.0008**	**0.79 (0.54, 1.04) p=0.0005**	**1.72 (1.57, 1.87) p<0.0001**	**3.53 (3.15, 3.90) p<0.0001**
	Keyboard	**0.24 (0.04, 0.44) p=0.0419**	**0.30 (0.05, 0.56) p=0.0446**	**0.40 (0.30, 0.51) p<0.0001**	**1.14 (1.00, 1.27) p<0.0001**	**2.86 (2.71, 3.02) p<0.0001**
	Painted Joint Tape	**2.50 (2.22, 2.78) p<0.0001**	**2.76 (2.54, 2.99) p<0.0001**	1.79 (0.10, 3.47) p=0.0706	**>6.14 (3/5) p=0.0079**	**>7.62 (5/5) p=0.0079**
Y. pestis	Aluminum	**0.68 (0.18, 1.19) p=0.0146**	**0.73 (0.30, 1.16) p=0.0040**	**>6.02 (2/5) p=0.0079**	**>6.75 (2/5) p=0.0079**	**>7.45 (4/5) p=0.0079**
	Carpet	**0.70 (0.51, 0.89) p=0.0007**	**4.49 (2.20, 6.78) p=0.0106**	**>6.89 (2/5) p=0.0079**	**>7.98 (5/5) p=0.0079**	**>7.98 (5/5) p=0.0079**
	Keyboard	0.48 (-0.01, 0.96) p=0.0833	**0.63 (0.27, 1.00) p=0.0138**	**3.70 (3.30, 4.10) p<0.0001**	**>7.32 (3/5) p=0.0079**	**>7.91 (5/5) p=0.0079**
	Painted Joint Tape	**2.80 (2.02, 3.58) p<0.0001**	**>5.80 (2/5) p=0.0079**	**>4.60 (1/5) p=0.0079**	**>6.86 (3/5) p=0.0079**	**>6.41 (2/5) p=0.0079**

* Mean log reduction is the mean of the base-10 logarithm of recovered agent from the control (time zero) coupons minus the mean of the base-10 logarithm of recovered agent from the persistence time coupons. A 95 % CI for the difference is shown in parentheses. A p-value is provided for the probability that the time zero and persistence time recoveries are the same. The p-value is from the two sample t-test with Satterthwaite's method to allow for potentially different variances in the two groups. P-values less than 0.05 denote less than 1 in 20 chance that a difference as large as or larger than observed would occur by chance if the time zero and persistence time means were truly identical. Comparisons with p-values less than 0.05 (statistically significant at the 0.05 level) are bolded.

† One or more of the persistence time coupons had no recovered agent. The mean log reduction of the form ">X" is calculated as the mean of the base-10 logarithm of recovered agent from the control (time zero) coupons minus the mean of the base-10 logarithm of recovered agent from the persistence time coupons except that "zero recovery" coupons have a substituted recovered value of "1" (base-10 log is 0). Since the log becomes an increasing negative value below 1 and is undefined at 0, this substitution is necessary and results in a lower bound on the mean log difference, as indicated by the ">". The number of "zero recovery" persistence time coupons and the total number of persistence time coupons is shown in parentheses. The p-value is from the non-parametric Kolmogorov-Smirnov test. P-values less than 0.05 denote less than 1 in 20 chance that results as different as or more different than observed would occur by chance if the distribution of the time zero and persistence time recoveries were truly identical. Comparisons with p-values less than 0.05 (statistically significant at the 0.05 level) are bolded.

5.0 Fumigation Technologies, Test Matrices, and Results

The intent of the fumigant testing was to assess the ability of the technology or decontamination process to decontaminant materials at conditions consistent with use in a facility. However, laboratory testing may present a challenge when testing at a smaller scale than for which the decontamination equipment was designed. For the Sabre ClO_2 testing, Sabre Technical Service, LLC. provided a prototype unit designed for reproducing their process in a smaller, lab-scale, environment (e.g., 317 L glove box). For the BIOQUELL hydrogen peroxide fumgition, the initial intent was to test using the 317 L glove box. In order to represent a typical room fumigation with the BIOQUELL hydrogen peroxide fumigation process, the temperature rise in the enclosed space due to the fumigation equipment must be minimized. To accomplish this in lab testing, BIOQUELL provided their Clarus S unit desgined for typical use in biological safety cabinets. After testing with that unit, it was decided to test at a larger scale (1275 L Biological Safety Cabinet), utilizing one of their larger fumigation units (Clarus C) with an attempt to obtain a better representation of room-scale fumigation. The STERIS VHP® system was a unit of similar size and design parameters to the BIOQUELL Clarus C, and tested at the same scale (1275 L).

Various controls were included in all of the fumigation testing described below. Application controls, positive controls, and blanks were included with the test samples in the experiments. An application control (i.e., spike amount) is a quantification of the amount of biological agent applied using a streak plate method described in Section 2.6. A positive control is a coupon spike with biological agent but not subjected to the test conditions. A laboratory blank is a coupon spiked with diluents without biological agent and not subjected to the test condition. A procedural blank is a coupon spiked with diluents without biological agent and subjected to the test condition. A test coupon is spiked with biological agent and subjected to the test condition.

No viable organisms were recovered from any blank coupon.

5.1 ClO_2 Fumigation (Sabre)

5.1.1 Description of Sabre ClO_2 Technology

The Sabre technology (Sabre Technical Services, LLC, Slingerlands, NY) in this evaluation uses ClO_2 as the active ingredient. ClO_2 is unstable as a compressed gas and, therefore, ClO_2 gas must be produced on-site. The Sabre decontamination technology includes the equipment and chemicals for on-site generation, delivery, removal, and neutralization of ClO_2. The decontamination technology was operated as specified in SOP MREF X-135[12] and summarized below.

The Sabre equipment included a 20 cm base onto which was mounted a 15 cm square, 91 cm high sparging column (see Figure 5-1). A 5-gallon container containing 15 L of an aqueous solution of 3 g/L of ClO_2 plus 1,000 parts per million (ppm) of chlorite was prepared on-site. The 5-gallon container was vented through a sodium thiosulfate trap and placed in an over-pack for safety. The ClO_2 solution was pumped (using a peristaltic pump) into a sparging column and air from the test chamber was pumped into and through the column to sparge ClO_2 from the liquid into the air stream. The air stream re-entered the glove box to establish the desired gaseous ClO_2 concentration. Liquid introduction from the reservoir of ClO_2/chlorite solution to the sparging column was initially at the rate of 60 mL per min. When the desired ClO_2 concentration in the test chamber was achieved, the liquid introduction into the sparging column was decreased to 0 to 3 mL per min. As the ClO_2 concentration dropped, additional gas was added to the chamber by manually increasing the flow rate to achieve the target concentration. The spent liquid exiting the sparging column was collected in a reservoir. The air from the chamber was recirculated into and out of the sparging column.

Figure 5-1. Sabre Bench-scale ClO_2 Generator.

At the end of the decontamination test the ClO_2 in the system was neutralized by pumping a 10% sodium hydroxide solution (or a 10% sodium thiosulfate solution) into the sparging column.

The concentration of ClO_2 in the test chamber was monitored before and during (approximately every 20 min) an experiment using a modified titration method based on the Standard Method 4500-ClO_2 E Amperometric Method II[13] as recommended and used by Sabre Technical Services and as specified in the test/QA plan. For this titration method, air from the test chamber is drawn through impingers (at a rate of 1 L/min using an air mass flow controller) that contain 15 mL of 5% potassium iodide in phosphate buffer (pH 7.0) solution. The pH of the impinger solution was measured and recorded before use. Under these conditions chlorine dioxide oxidizes iodide to iodine and reduces chlorine dioxide gas to the chlorite ion which dissolves in solution. Chlorite ion does not react at neutral pH. After collection and reaction of the chlorine dioxide gas, the impinger solution was acidified and the chlorite was allowed to react further with the iodide ion, forming additional iodine and reducing the chlorite to chloride. The pH of the impinger solution was measured and recorded immediately before titration. The total resulting iodine was reduced to iodide when titrated against standard 0.1 normal [equal to 0.1 molar] sodium thiosulfate (STS). Molecular iodine appears yellow-brown in aqueous solution. The titration endpoint was determined when the color of the solution changed from yellow-brown to colorless. The volume (mL) of STS solution titrated is proportional to the amount of iodine generated, which is proportional to the gas-phase chlorine dioxide concentration in the air that passed through the impinger. Using the formula in Equation 9 below, the concentration of chlorine dioxide was calculated.

Equation 9. $$ClO_2\ (\text{ppmv}) = \frac{V_1(mL)\times M}{V_2(L)}\times\frac{1}{5}\times 24.45\times 1000$$

where:

ClO_2 = chlorine dioxide (ppmv in air)
V_1 = volume of STS titrant (mL)
M = molarity (mol/L) of STS titrant (which for STS is equal to its normality)
V_2 = volume of air (at 25 °C, 1 atm) that passed through impinger (L)
24.45 = ideal gas constant, L/mol, at 25 °C, 1 atm
1000 = conversion factor = 10^6 ppmv x 1 L / 1000 mL

Certified NIST- traceable chlorite standards, appropriately diluted in solution comparable to the sampling solution, were titrated each day of chlorine dioxide sampling to verify accuracy.

5.1.2 Test Matrix for Sabre ClO_2 Fumigation

The tests performed with Sabre ClO_2 are shown in Table 5-1. The experimental design tested decontamination efficacy by determining whether there was a difference between the log reductions in the viable biological agents after fumigation compared to controls for aluminum, keyboard, carpet and joint tape. These tests also assessed whether there were any differences in efficacy at varying RH levels and varying fumigation contact times. Critical parameters impacting the viability of biological agents included ClO_2 concentration, fumigation contact time, temperature, RH, and natural attenuation. An adaptive management approach was used to incorporate new knowledge into the testing as decontamination efficacy results became available.

Table 5-1. Test Matrix for Sabre ClO_2 Fumigation

Biological Agent	Material	ClO_2 Concentration, Temperature	% RH ± % (full scale)	Contact Times (min)
B. anthracis + Biological indicator*	Aluminum, Keyboard Carpet, Joint tape	3,000 ppmv, 23 °C ± 2 °C	40% ± 5%	0, 20, 40, 60, 90, 180
			75% ± 5%	0, 20, 40, 60, 90, 180
B. suis	Aluminum, Keyboard Carpet, Joint tape	50-100 ppmv ± 25 ppmv, 23 °C ± 2 °C	40% ± 5%	0, 30, 60, 120
			60% ± 5%	0, 30, 60, 120
			75% ± 5%	0, 30, 60, 120
F. tularensis	Aluminum, Keyboard Carpet, Joint tape	50-100 ppmv ± 25 ppmv, 23 °C ± 2 °C	40% ± 5%	0, 30, 60, 120
			75% ± 5%	0, 30, 60, 120
Vaccinia virus	Aluminum†, Keyboard Carpet†, Joint tape†	50-100 ppmv ± 25 ppmv, 23 °C ± 2 °C	40% ± 5%	0, 60, 120
			60% ± 5%	0, 120
			75% ± 5%	0, 30, 60, 120†
Y. pestis	Aluminum, Keyboard Carpet, Joint tape	50-100 ppmv ± 25 ppmv, 23 °C ± 2 °C	40% ± 5%	0, 30, 60, 120
			75% ± 5%	0, 30, 60, 120

* Five *B. atrophaeus* on steel in Tyvek® packaging were exposed to Sabre ClO_2 fumigation at the 40% RH at each contact time out to 180 min.
† Aluminum, carpet, and joint tape were not tested at 120 min at 75% RH.

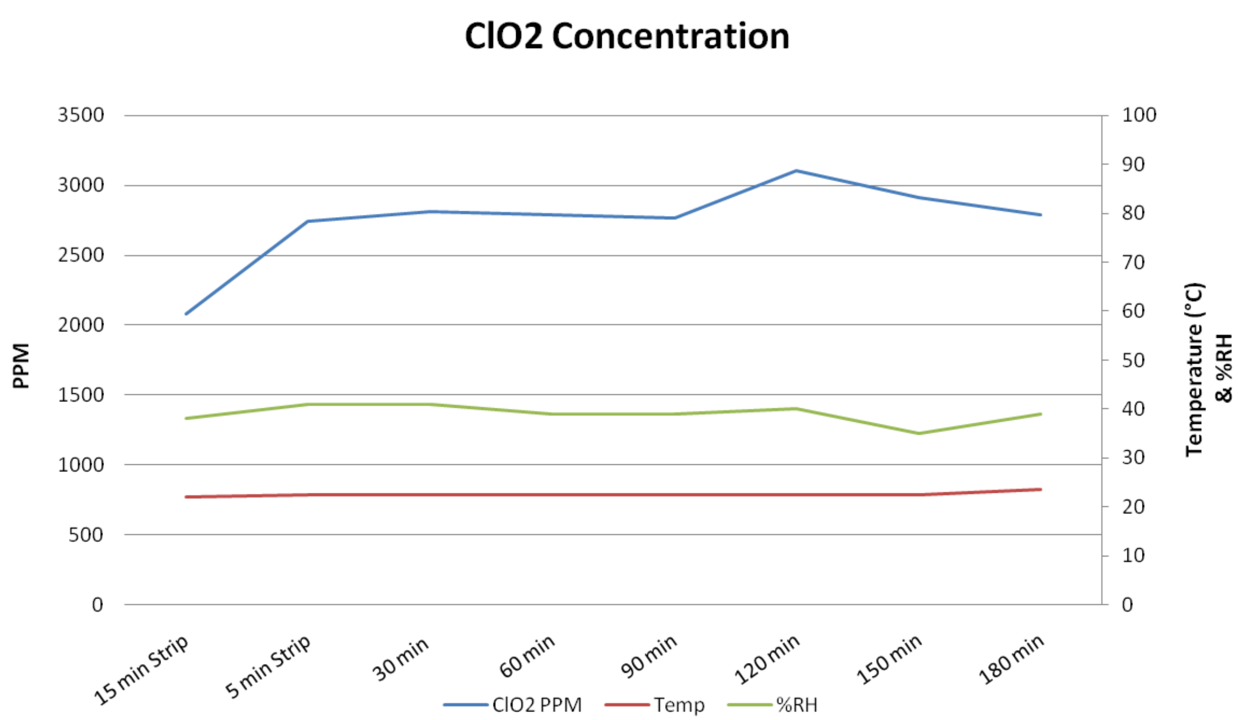
Five replicate test coupons (plus one procedural blank) and five replicate positive control coupons (plus one laboratory blank) were included at each set of conditions and time points. Negative controls (procedural blanks and laboratory blanks) were coupons to which corresponding diluent, but no biological agent, was applied.

Decontamination was halted by dropping the atmospheric concentration of ClO_2 to near zero. The coupons were placed in sealed vials and transferred into a second BSC III, the vials were opened, and the coupons were transferred into the extraction medium. No quenching agents were added to stop the decontamination reaction because residual ClO_2 was assumed to be rapidly removed from the coupon through convection, diffusion, and dilution.

5.1.3 Sabre ClO_2 Fumigation Results

Figure 5-2 shows a graph of typical temperature, RH, and ClO_2 concentration during a fumigation cycle.

Figure 5-2. Temperature, RH, and ClO_2 Concentration Dynamics During aTypical Fumigation Cycle; “15 min Strip” and “5 min Strip” Refers to Addition of ClO_2 to the Test Chamber.



B. atrophaeus

Biological indicators that were *B. atrophaeus* spores (nominally 106 spores) on steel in Tyvek® packaging were exposed during the Sabre ClO_2 fumigation test with *B. anthracis* spores at "3,000 ppmv ClO_2, 23°C, 40% RH". All biological indicators, including five replicates at the 180-min contact time (the longest time tested), were positive for growth, which is indicative of incomplete kills. The biological indicators (on steel) are consistent with *B. anthracis* on aluminum which retained viable spores at all treatment conditions.

B. anthracis

Sabre ClO_2 fumigation results for *B. anthracis* spores are presented in Table 5-2 and Figures 5-3 and 5-4. For all materials tested except aluminum, a 90-min contact time at 3,000 ppmv ClO_2, 23 °C, and 75% RH resulted in no viable *B. anthracis* spore recovery. *B. anthracis* spores were recovered from aluminum following Sabre ClO_2 fumigation for all tests conducted (out to 180 min).

Log reductions in *B. anthracis* spores on keyboard were ≥ 6.57 for all tests conducted at both 40% and 75% RH. Viable *B. anthracis* spores (6.6 CFUs/coupon) were recovered from keyboard at only one condition (20-min contact time, 3,000 ppmv ClO_2, 23 °C, and 75% RH).

At the 3,000 ppmv ClO_2, 23 °C condition, no viable *B. anthracis* spores were recovered from carpet after the 180-min contact time at 40% RH or following the 90-min contact time at 75% RH.

At the 3,000 ppmv ClO_2, 23 °C condition, no viable *B. anthracis* spores were recovered from joint tape after the 90-min contact time at 75% RH. At the corresponding test condition except 40% RH rather than 75% RH, *B. anthracis* spores were recovered at 180-min contact time (the longest time tested).

Table 5-2. Sabre ClO_2 Fumigation Results for *B. anthracis*

Contact Time	Material	Spike Amount CFU/ coupon	Mean Recovered *B. anthracis* (CFU/coupon)* Positive Control†	Test Coupon‡	Mean Log Reduction*
3,000 ppmv ClO_2, 23°C, 40% RH					
0 min	Aluminum	9.60×10^6	$2.31 \pm 0.07 \times 10^6$	Not applicable	Not applicable
	Keyboard	1.00×10^7	$2.96 \pm 0.31 \times 10^6$	Not applicable	Not applicable
	Carpet	1.00×10^7	$5.91 \pm 0.40 \times 10^6$	Not applicable	Not applicable
	Joint tape	9.60×10^6	$2.42 \pm 0.10 \times 10^6$	Not applicable	Not applicable
20 min	Aluminum	9.60×10^6	$3.84 \pm 0.78 \times 10^6$	$2.82 \pm 1.15 \times 10^6$	0.17 ± 0.23
	Keyboard	1.00×10^7	$3.71 \pm 1.23 \times 10^6$	0.00 ± 0.00	6.57 ± 0.00
	Carpet	1.00×10^7	$5.53 \pm 1.20 \times 10^6$	$5.16 \pm 3.13 \times 10^4$	2.22 ± 0.61
	Joint tape	9.60×10^6	$3.80 \pm 0.37 \times 10^6$	$4.21 \pm 3.33 \times 10^3$	3.28 ± 0.80
40 min	Aluminum	9.60×10^6	$3.84 \pm 0.78 \times 10^6$	$9.01 \pm 2.48 \times 10^5$	0.64 ± 0.12
	Keyboard	1.00×10^7	$3.71 \pm 1.23 \times 10^6$	0.00 ± 0.00	6.57 ± 0.00
	Carpet	1.00×10^7	$5.53 \pm 1.20 \times 10^6$	$8.89 \pm 4.49 \times 10^3$	2.84 ± 0.24
	Joint tape	9.60×10^6	$3.80 \pm 0.37 \times 10^6$	$9.50 \pm 4.24 \times 10^3$	2.65 ± 0.26
60 min	Aluminum	9.60×10^6	$3.84 \pm 0.78 \times 10^6$	$1.19 \pm 0.89 \times 10^6$	0.58 ± 0.25
	Keyboard	1.00×10^7	$3.71 \pm 1.23 \times 10^6$	0.00 ± 0.00	6.57 ± 0.00
	Carpet	1.00×10^7	$5.53 \pm 1.20 \times 10^6$	$1.20 \pm 1.39 \times 10^2$	5.41 ± 1.24
	Joint tape	9.60×10^6	$3.80 \pm 0.37 \times 10^6$	$1.07 \pm 2.07 \times 10^4$	3.29 ± 0.96
90 min	Aluminum	9.60×10^6	$3.84 \pm 0.78 \times 10^6$	$2.61 \pm 3.26 \times 10^5$	1.54 ± 0.67
	Keyboard	1.00×10^7	$3.71 \pm 1.23 \times 10^6$	0.00 ± 0.00	6.57 ± 0.00
	Carpet	1.00×10^7	$5.53 \pm 1.20 \times 10^6$	$2.66 \pm 4.91 \times 10^2$	5.67 ± 1.49
	Joint tape	9.60×10^6	$3.80 \pm 0.37 \times 10^6$	$1.22 \pm 1.47 \times 10^4$	2.68 ± 0.42
180 min	Aluminum	9.60×10^6	$3.84 \pm 0.78 \times 10^6$	$1.21 \pm 2.39 \times 10^3$	4.88 ± 1.66
	Keyboard	1.00×10^7	$3.71 \pm 1.23 \times 10^6$	0.00 ± 0.00	6.57 ± 0.00
	Carpet	1.00×10^7	$5.53 \pm 1.20 \times 10^6$	0.00 ± 0.00	6.74 ± 0.00
	Joint tape	9.60×10^6	$3.80 \pm 0.37 \times 10^6$	$8.53 \pm 11.1 \times 10^3$	2.93 ± 0.55

Contact Time	Material	Spike Amount CFU/ coupon	Mean Recovered *B. anthracis* (CFU/coupon)*		Mean Log Reduction*
			Positive Control†	Test Coupon‡	
3,000 ppmv ClO_2, 23ºC, 75% RH					
20 min	Aluminum	5.73 x 10^6 §	7.92 ± 1.08 x 10^6 #	1.13 ± 1.88 x 10^2	5.95 ± 1.32
	Keyboard	3.83 x 10^6 §	8.91 ± 1.09 x 10^6 #	6.60 ± 14.8 x 10^0	6.65 ± 0.68
	Carpet	5.73 x 10^6 §	3.89 ± 0.47 x 10^6	9.99 ± 7.43 x 10^3	2.68 ± 0.31
	Joint tape	3.83 x 10^6 §	3.89 ± 4.21 x 10^6	1.07 ± 2.03 x 10^2	5.69 ± 1.27
40 min	Aluminum	5.73 x 10^6	6.64 ± 0.77 x 10^6	3.14 ± 4.46 x 10^2	4.97 ± 1.15
	Keyboard	3.83 x 10^6 §	1.41 ± 0.77 x 10^7 #	0.00 ± 0.00	7.15 ± 0.00
	Carpet	5.73 x 10^6 §	4.45 ± 1.65 x 10^6	2.39 ± 2.34 x 10^3	3.40 ± 0.35
	Joint tape	3.83 x 10^6 §	5.12 ± 1.44 x 10^6 #	2.00 ± 2.99 x 10^1	6.04 ± 0.92
60 min	Aluminum	5.73 x 10^6 §	6.98 ± 1.07 x 10^6 #	9.01 ± 18.8 x 10^2	4.99 ± 1.29
	Keyboard	3.83 x 10^6 §	8.99 ± 0.48 x 10^6 #	0.00 ± 0.00	6.95 ± 0.00
	Carpet	5.73 x 10^6 §	3.79 ± 0.16 x 10^6	2.20 ± 2.56 x 10^2	4.52 ± 0.57
	Joint tape	3.83 x 10^6 §	2.54 ± 1.06 x 10^6	4.02 ± 3.67 x 10^1	5.31 ± 1.00
90 min	Aluminum	5.73 x 10^6 §	6.27 ± 0.76 x 10^6	3.73 ± 6.45 x 10^2	5.35 ± 1.44
	Keyboard	3.83 x 10^6 §	9.07 ± 1.37 x 10^6 #	0.00 ± 0.00	6.96 ± 0.00
	Carpet	5.73 x 10^6 §	3.72 ± 0.66 x 10^6	0.00 ± 0.00	6.57 ± 0.00
	Joint tape	3.83 x 10^6 §	3.61 ± 0.31 x 10^6	0.00 ± 0.00	6.56 ± 0.00
180 min	Aluminum	5.73 x 10^6 §	7.16 ± 1.15 x 10^6	2.00 ± 2.99 x 10^1	6.19 ± 0.92
	Keyboard	3.83 x 10^6 §	1.66 ± 1.46 x 10^7 #	0.00 ± 0.00	7.22 ± 0.00
	Carpet	5.73 x 10^6 §	5.99 ± 1.11 x 10^6	0.00 ± 0.00	6.78 ± 0.00
	Joint tape	3.83 x 10^6 §	3.16 ± 0.18 x 10^6	0.00 ± 0.00	6.50 ± 0.00

* Data are expressed as mean ± standard deviation of five replicates.
† Positive control coupons were spiked but not exposed to the fumigant.
‡ Test coupons were spiked and exposed to the fumigant for the contact time.
§ Application lower than the target range 7.5 x 10^6 - 1.25 x 10^7 CFU/coupon.
\# Exceeds target recovery of ≤120% of spike amount.

Figure 5-3. Sabre fumigation results for *B. anthracis* at 3,000 ppmv ClO_2 and 23 °C, line chart.

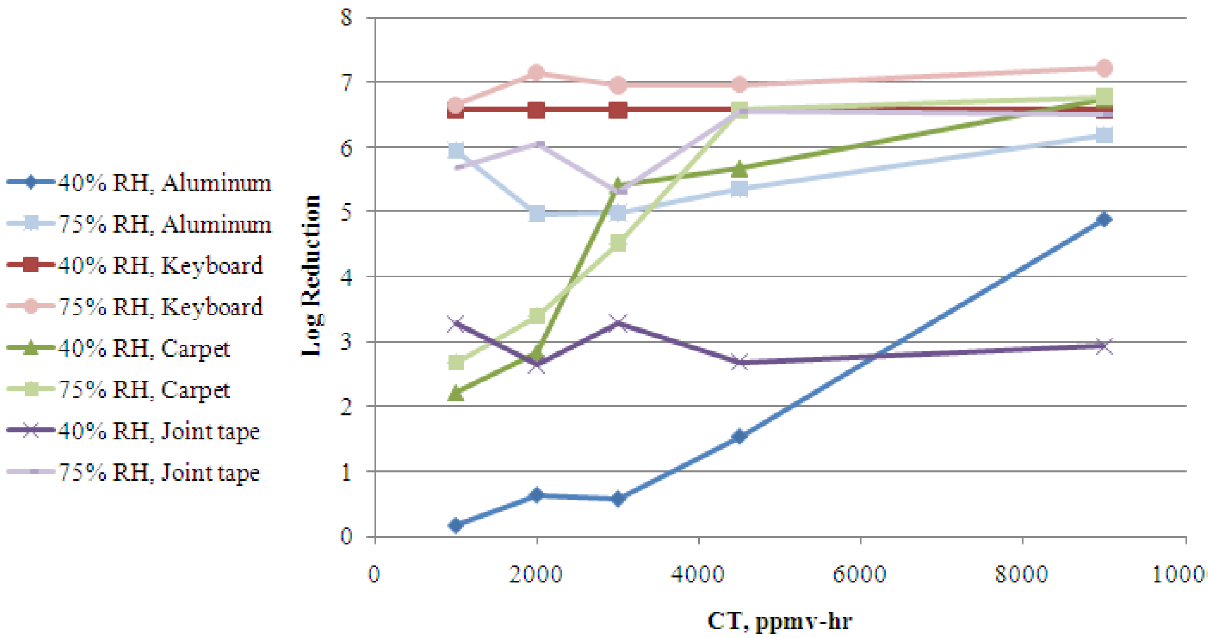


Figure 5-4. Sabre Fumigation Results for *B. anthracis* at 3,000 ppmv ClO_2 and 23 °C, Column Chart.

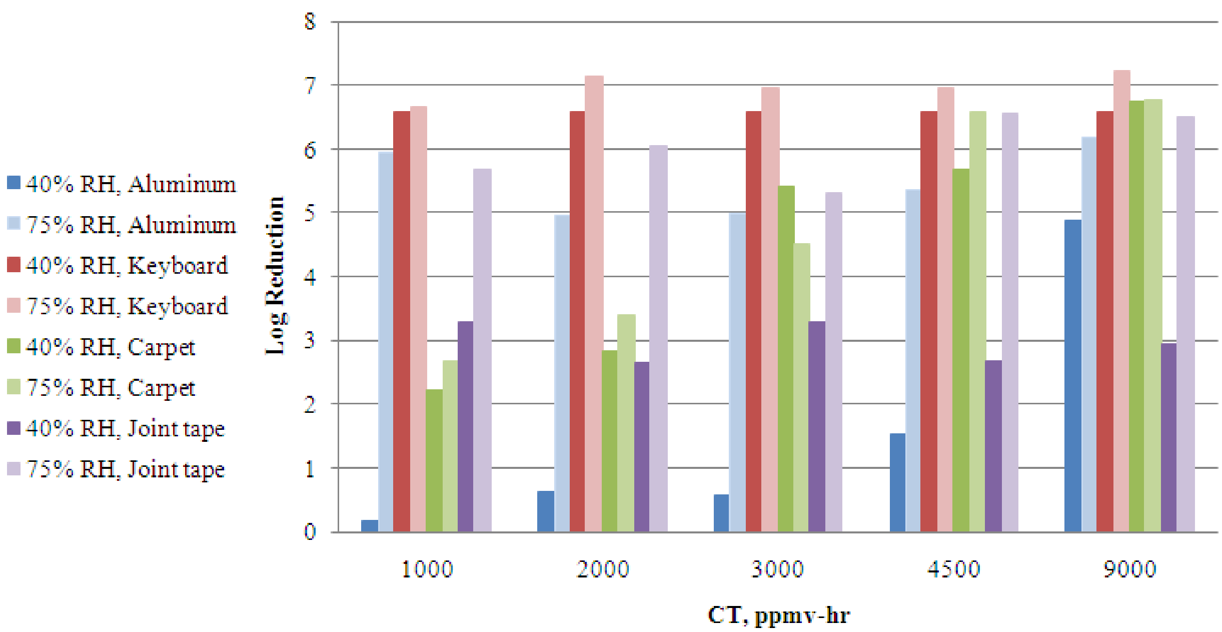


Table 5-3 provides a summary of ClO_2 decontamination efficacy against *B. anthracis* spores, calculated as the difference in the mean log of viable spores recovered from coupons at time zero and the mean log of viable spores recovered from coupons after fumigation for a given contact time. The 95% CI and p-value are also shown. Significant reduction in viable biological agent was observed for all agent/material combinations with a 40-min contact time at both 40% RH and 75% RH.

Table 5-3. Summary of Efficacy (Calculated as Mean Log Reduction) of ClO_2 against *B. anthracis* spores

		Mean Log Reduction (95% CI) and p-value* **or Mean Log Reduction (# of treated coupons with zero recovery/# of treated coupons) and p-Value†**				
Material	**% RH**	**20 min**	**40 min**	**60 min**	**90 min**	**180 min**
Aluminum	40	0.17 (-0.085, 0.42) p=0.1865	**0.64 (0.49, 0.78) p<0.0001**	**0.57 (0.30, 0.85) p=0.0052**	**1.54 (0.84, 2.24) p=0.0066**	**>4.87 (2/5) p=0.0079**
	75	**>5.94 (3/5) p=0.0079**	**>4.97 (1/5) p=0.0079**	**>4.98 (1/5) p=0.0079**	**>5.35 (2/5) p=0.0079**	**>6.18 (3/5) p=0.0079**
Carpet	40	**2.21 (1.57, 2.84) p=0.0010**	**2.83 (2.56, 3.11) p<0.0001**	**>5.40 (2/5) p=0.0079**	**>5.66 (3/5) p=0.0079**	**>6.73 (5/5) p=0.0079**
	75	**2.68 (2.36, 3.01) p<0.0001**	**3.38 (2.99, 3.77) p<0.0001**	**4.51 (3.92, 5.11) p<0.0001**	**>6.57 (5/5) p=0.0079**	**>6.77 (5/5) p=0.0079**
Keyboard	40	**>6.55 (5/5) p=0.0079**	**>6.55 (5/5) p=0.0079**	**>6.55 (5/5) p=0.0079**	**>6.55 (5/5) p=0.0079**	**>6.55 (5/5) p=0.0079**
	75	**>6.64 (4/5) p=0.0079**	**>7.11 (5/5) p=0.0079**	**>6.95 (5/5) p=0.0079**	**>6.95 (5/5) p=0.0079**	**>7.07 (5/5) p=0.0079**
Joint Tape	40	**3.28 (2.45, 4.11) p=0.0008**	**2.65 (2.38, 2.93) p<0.0001**	**3.29 (2.30, 4.28) p=0.0015**	**2.68 (2.25, 3.11) p=0.0001**	**2.93 (2.36, 3.49) p=0.0003**
	75	**>5.51 (3/5) p=0.0079**	**>6.02 (3/5) p=0.0079**	**>5.27 (2/5) p=0.0079**	**>6.56 (5/5) p=0.0079**	**>6.50 (5/5) p=0.0079**

* Mean log reduction is the mean of the base-10 logarithm of recovered agent from the control coupons minus the mean of the base-10 logarithm of recovered agent from the treated coupons. A 95 % CI for the difference is shown in parentheses. A p-value is provided for the probability that the control and treatment recoveries are the same. The p-value is from the two sample t-test with Satterthwaite's method to allow for potentially different variances in the two groups. p-Values less than 0.05 denote less than 1 in 20 chance that a difference as large as or larger than observed would occur by chance if the control and treatment means were truly identical. Comparisons with p-values less than 0.05 (statistically significant at the 0.05 level) are bolded.

† One or more of the treatment coupons had no recovered agent. The mean log reduction of the form ">X" is calculated as the mean of the base-10 logarithm of recovered agent from the control coupons minus the mean of the base-10 logarithm of recovered agent from the treated coupons except that "zero recovery" coupons have a substituted recovered value of "1" (base-10 log is 0). Since the log becomes an increasingly negative value below 1 and is undefined at 0, this substitution is necessary and results in a lower bound on the mean log difference, as indicated by the ">". The number of "zero recovery" treatment coupons and the total number of treatment coupons is shown in parentheses. The p-value is from the non-parametric Kolmogorov-Smirnov test. p-Values less than 0.05 denote less than 1 in 20 chance that results as different as or more different than observed would occur by chance if the distribution of the control and treatment recoveries were truly identical. Comparisons with p-values less than 0.05 (statistically significant at the 0.05 level) are bolded.

B. suis

Sabre ClO_2 fumigation results for *B. suis* are presented in Tables 5-4 and 5-5 and in Figures 5-5 and 5-6. Persistence tests reported in Section 4.2.1, as well as positive controls used in these tests, showed that there was a differential loss of viability of *B. suis*, depending on the coupon type. Specifically, over the 1 hr drying time and multiple hr contact times, there was a significant reduction in viable *B. suis* on carpet and joint tape. As described in the Introduction, the calculated log reductions reflect the incremental impact of the fumigation technology.

B. suis on aluminum exposed to 50-100 ppmv ClO_2 (23 °C) exhibited log reductions that increased with increasing RH. Specifically, log reductions were low (<2.0) for all contact times tested at low RH (40%). At the same ClO_2 concentration and temperature, the log reductions increased but remained <4.0 at 60% RH. At the same ClO_2 concentration and temperature, the log reductions were >5.0 with no *B. suis* recovered from aluminum after 60-min and 120-min contact times at 75% RH.

B. suis on keyboard exposed to 50-100 ppmv ClO_2 (23 °C) exhibited low log reductions at both 40% and 60 % RH (<1.0) for all contact times tested. At the same ClO_2 concentration and temperature, the log reductions were low (≤2.66 up to 60 min), but reached a 5.63 log reduction at the 120-min contact time at 75% RH. *B. suis* was recovered from every test conducted with keyboard. Because of their small size, the keyboard keys were spiked with a single 100 µL droplet, rather than the 10 x 10 µL droplets used with all of the other materials. The single droplet may result in a "stacking" of bacteria that may shield them from contact with decontamination treatments, thus resulting in relatively lower efficacy results.

There was generally a decrease in the recovery of viable *B. suis* from carpet positive control coupons (not exposed to fumigation) during the period of decontamination. The decline in viable *B. suis* from carpet was consistent with the ~2 log loss of viable *B. suis* from carpet after 2 to 4 hr observed in the persistence testing. During fumigation testing with ClO_2, the positive control coupons were allowed to dry for one hr,and an additional period of time (about 30 min) passes before the CT clock starts for fumigation. Thus, after 120-min fumigation, the positive controls may be at a time equivalent to 3.5 hr in the persistence testing. There is a decrease in the recovery of viable *B. suis* from carpet positive control coupons (not exposed to fumigation) during the period of decontamination that was consistent with the loss of viable *B. suis* from carpet in the persistence testing.

Because the log reduction reflects only the incremental impact of fumigation (controls for loss of viability without treatment), overall efficacy must be interpreted in the context of the loss of viability with treatment. For example, carpet exposed to the fumigation treatment for 60 min at 60% RH shows a log reduction of only 2.11. However, only about 10^2 bacteria are recovered from the untreated carpet. (This recovery results in a very low base to which the treated carpet was compared.) Compared to the amount of bacteria spiked onto the coupon (3.77×10^7 CFUs), the amount of bacteria recovered after the 60-min treatment (6.00×10^1 CFUs/coupon) represents almost a 6 log reduction in viable bacteria attributable to the fumigation and the loss of viability from carpet arising from other (unknown) causes.

There was a decrease in the recovery of viable *B. suis* from joint tape positive control coupons (not exposed to fumigation) during the period of decontamination. (The decline in viable *B. suis* from joint tape was consistent with the >5 log loss of viable *B. suis* from joint tape after 2 hr observed in the persistence testing.) *B. suis* was only recovered from joint tape after Sabre ClO_2 fumigation from "50-100 ppmv ClO_2, 23 °C, 40% RH" at contact times of 30 min and 60 min; the associated log reductions were <2.0. Compared to the amount of bacteria spiked onto the coupon (5.90×10^7 CFUs for 40% RH test and 8.23×10^7 CFUs for 60% RH test), the amount of bacteria recovered after the 120-min treatment (0 CFUs/coupon) represents >7.7 log reduction in viable bacteria attributable to the fumigation and the loss of viability from joint tape arising from other (unknown) causes.

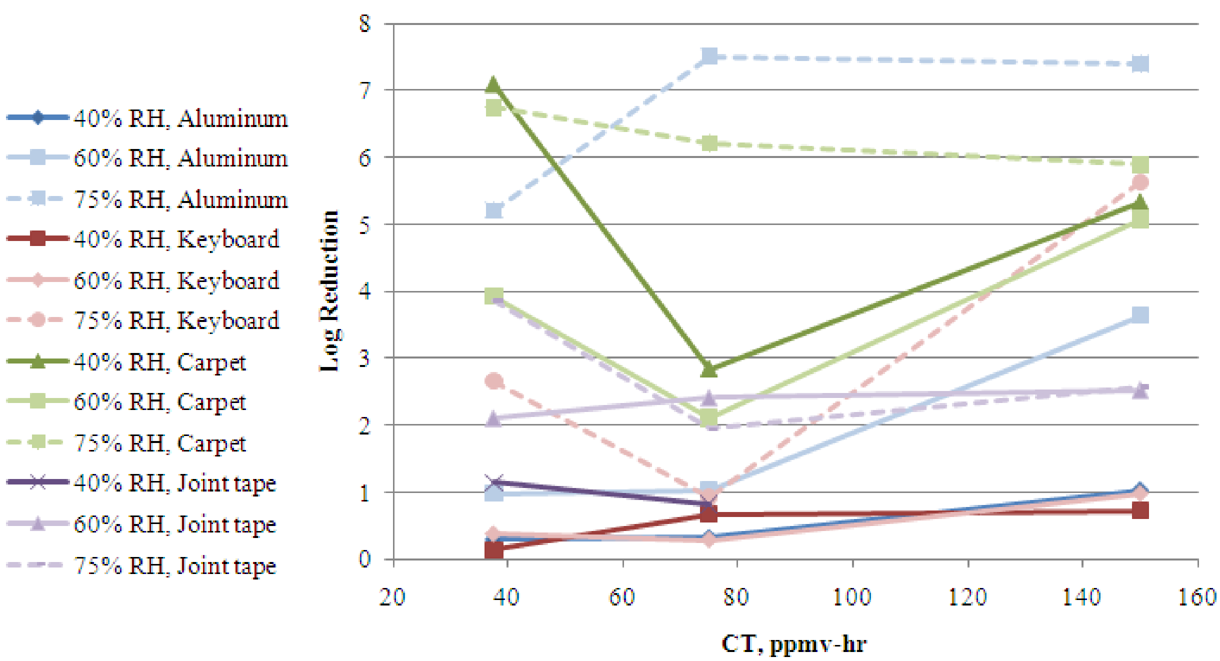
Table 5-4. Sabre ClO_2 Fumigation Results for *B. suis*

Contact Time	Material	Spike Amount (CFU/coupon)	Mean Recovered *B. suis* (CFU/coupon)*		Mean Log Reduction*
			Positive Control†	Test Coupon‡	
50-100 ppmv ClO_2, 23°C, 40% RH					
0 min	Aluminum	5.90×10^7	$4.65 \pm 0.97 \times 10^7$	Not applicable	Not applicable
	Keyboard	5.90×10^7	$3.62 \pm 1.26 \times 10^7$	Not applicable	Not applicable
	Carpet	5.90×10^7	$7.29 \pm 0.82 \times 10^7$	Not applicable	Not applicable
	Joint tape	5.90×10^7	$1.08 \pm 0.35 \times 10^4$§	Not applicable	Not applicable
30 min	Aluminum	5.90×10^7	$7.37 \pm 1.24 \times 10^7$	$3.83 \pm 0.38 \times 10^7$	0.29 ± 0.04
	Keyboard	5.90×10^7	$3.38 \pm 1.11 \times 10^7$	$2.47 \pm 0.48 \times 10^7$	0.14 ± 0.10
	Carpet	5.90×10^7	$2.47 \pm 1.01 \times 10^7$	$6.60 \pm 14.8 \times 10^0$	7.09 ± 0.68
	Joint tape	5.90×10^7	$4.40 \pm 2.39 \times 10^2$§	$2.51 \pm 3.94 \times 10^3$	1.14 ± 2.06
60 min	Aluminum	3.37×10^7	$3.38 \pm 0.43 \times 10^7$	$1.61 \pm 0.16 \times 10^7$	0.32 ± 0.05
	Keyboard	3.37×10^7	$4.55 \pm 1.81 \times 10^7$	$1.01 \pm 0.27 \times 10^7$	0.67 ± 0.13
	Carpet	3.37×10^7	$5.53 \pm 2.67 \times 10^5$	$2.55 \pm 4.67 \times 10^3$	2.84 ± 0.67
	Joint tape	3.37×10^7	$1.74 \pm 1.30 \times 10^4$§	$4.16 \pm 4.11 \times 10^3$	0.82 ± 0.49
120 min	Aluminum	5.90×10^7	$8.18 \pm 2.48 \times 10^7$	$7.89 \pm 2.57 \times 10^6$	1.03 ± 0.12
	Keyboard	5.90×10^7	$2.94 \pm 0.21 \times 10^7$	$5.81 \pm 1.90 \times 10^6$	0.72 ± 0.14
	Carpet	5.90×10^7	$2.14 \pm 1.39 \times 10^5$	0.00 ± 0.00	5.33 ± 0.00
	Joint tape	5.90×10^7	0.00 ± 0.00§	0.00 ± 0.00	Not calculable
50-100 ppmv ClO_2, 23°C, 60% RH					
0 min	Aluminum	3.77×10^7	$8.85 \pm 10.9 \times 10^7$	Not applicable	Not applicable
	Keyboard	3.77×10^7	$1.17 \pm 0.15 \times 10^7$	Not applicable	Not applicable
	Carpet	3.77×10^7	$1.16 \pm 0.59 \times 10^7$	Not applicable	Not applicable
	Joint tape	3.77×10^7	$8.44 \pm 3.36 \times 10^3$§	Not applicable	Not applicable
30 min	Aluminum	8.23×10^7	$1.94 \pm 0.20 \times 10^7$	$2.08 \pm 0.55 \times 10^6$	0.98 ± 0.12
	Keyboard	8.23×10^7	$5.69 \pm 1.96 \times 10^7$	$2.53 \pm 0.79 \times 10^7$	0.37 ± 0.14
	Carpet	8.23×10^7	$4.94 \pm 3.37 \times 10^6$	$9.34 \pm 10.6 \times 10^2$	3.92 ± 0.45
	Joint tape	8.23×10^7	$1.28 \pm 1.47 \times 10^2$§	0.00 ± 0.00	2.11 ± 0.00
60 min	Aluminum	3.77×10^7	$1.19 \pm 0.17 \times 10^7$	$1.77 \pm 1.74 \times 10^6$	1.03 ± 0.48
	Keyboard	3.77×10^7	$7.91 \pm 0.67 \times 10^6$	$4.19 \pm 0.42 \times 10^6$	0.28 ± 0.04
	Carpet	3.77×10^7	$8.81 \pm 2.35 \times 10^2$	$6.00 \pm 10.1 \times 10^1$	2.11 ± 1.16
	Joint tape	3.77×10^7	$2.60 \pm 1.66 \times 10^2$§	0.00 ± 0.00	2.41 ± 0.00

Contact Time	Material	Spike Amount (CFU/coupon)	Mean Recovered *B. suis* (CFU/coupon)*		Mean Log Reduction*
			Positive Control†	Test Coupon‡	
120 min	Aluminum	8.23×10^{7}	$2.01 \pm 1.13 \times 10^{7}$	$4.77 \pm 1.26 \times 10^{3}$	3.64 ± 0.11
	Keyboard	8.23×10^{7}	$2.09 \pm 0.70 \times 10^{7}$	$2.25 \pm 0.49 \times 10^{6}$	0.98 ± 0.11
	Carpet	8.23×10^{7}	$1.17 \pm 1.01 \times 10^{5}$	0.00 ± 0.00	5.07 ± 0.00
	Joint tape	8.23×10^{7}	$3.31 \pm 2.75 \times 10^{2}$§	0.00 ± 0.00	2.52 ± 0.00
50-100 ppmv ClO_2, 23°C, 75% RH					
0 min	Aluminum	3.33×10^{7}	$1.28 \pm 0.89 \times 10^{7}$	Not applicable	Not applicable
	Keyboard	3.33×10^{7}	$4.32 \pm 2.10 \times 10^{7}$	Not applicable	Not applicable
	Carpet	3.33×10^{7}	$1.01 \pm 0.36 \times 10^{7}$	Not applicable	Not applicable
	Joint tape	3.33×10^{7}	$4.20 \pm 0.64 \times 10^{4}$§	Not applicable	Not applicable
30 min	Aluminum	3.33×10^{7}	$8.59 \pm 1.98 \times 10^{6}$	$6.87 \pm 10.9 \times 10^{2}$	5.21 ± 1.61
	Keyboard	3.33×10^{7}	$3.25 \pm 2.12 \times 10^{7}$	$2.12 \pm 1.78 \times 10^{5}$	2.66 ± 1.10
	Carpet	3.33×10^{7}	$5.54 \pm 3.25 \times 10^{6}$	0.00 ± 0.00	6.74 ± 0.00
	Joint tape	3.33×10^{7}	$7.29 \pm 5.09 \times 10^{3}$§	0.00 ± 0.00	3.86 ± 0.00
60 min	Aluminum	8.00×10^{7}	$3.22 \pm 0.64 \times 10^{7}$	0.00 ± 0.00	7.51 ± 0.00
	Keyboard	8.00×10^{7}	$6.31 \pm 1.73 \times 10^{7}$	$7.96 \pm 2.46 \times 10^{6}$	0.92 ± 0.14
	Carpet	8.00×10^{7}	$1.61 \pm 2.10 \times 10^{6}$	0.00 ± 0.00	6.21 ± 0.00
	Joint tape	8.00×10^{7}	$8.66 \pm 11.5 \times 10^{1}$§	0.00 ± 0.00	1.94 ± 0.00
120 min	Aluminum	3.33×10^{7}	$2.51 \pm 1.37 \times 10^{7}$	0.00 ± 0.00	7.40 ± 0.00
	Keyboard	3.33×10^{7}	$9.60 \pm 0.85 \times 10^{6}$	$8.63 \pm 19.2 \times 10^{3}$	5.63 ± 2.05
	Carpet	3.33×10^{7}	$7.81 \pm 16.7 \times 10^{5}$	0.00 ± 0.00	5.89 ± 0.00
	Joint tape	3.33×10^{7}	$3.80 \pm 3.62 \times 10^{2}$§	0.00 ± 0.00	2.58 ± 0.00

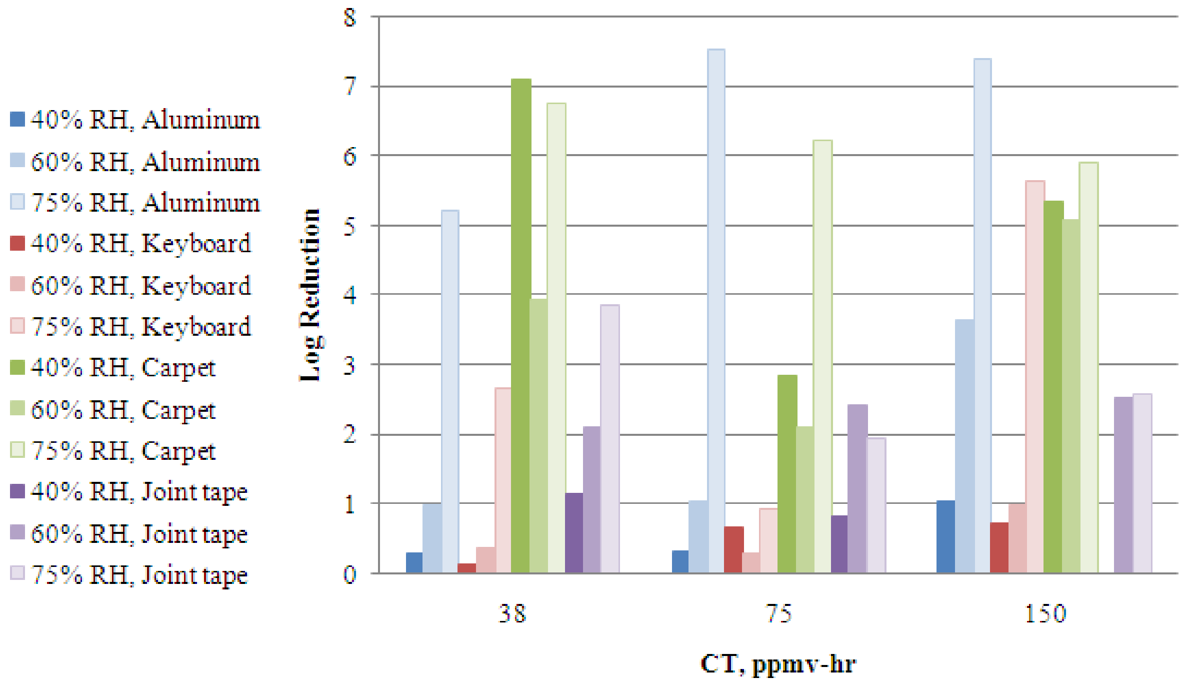
* Data are expressed as mean ± standard deviation of five replicates.
† Positive control coupons were spiked but not exposed to the fumigant.
‡ Test coupons were spiked and exposed to the fumigant for the contact time.
§ Below target recovery of ≥10% of spike amount.

Figure 5-5. Sabre Fumigation Results for *B. suis* at 50-100 ppmv ClO_2 and 23 °C, Line Chart.



* Log reduction not calculated for the 150 CT as *B. suis* was not recovered from either the test or associated control coupons.

Figure 5-6. Sabre Fumigation Results for *B. suis* at 50-100 ppmv ClO_2 and 23 °C, Column Chart.



* Log reduction not calculated for the 150 CT as *B. suis* was not recovered from either the test or associated control coupons.

Table 5-5 provides a summary of ClO_2 decontamination efficacy against *B. suis*, calculated as the difference in the mean log of viable bacteria recovered from positive control coupons and the mean log of viable bacteria recovered from coupons after fumigation for a given contact time. The 95% CI and p-value are also shown. To control for history, the elapsed time from spiking to recovery was the same for the positive control and test coupons. At the 120-min contact time efficacy was demonstrated at all three RH conditions (40%, 60%, and 75%) for aluminum, carpet, and keyboard. Because of the temporal loss of viable bacteria from positive control coupons at 40% and 120% RH, only the 60% RH results confirm incremental efficacy from the fumigation compared to the temporal loss in viability.

For aluminum and keyboard (hard, non-porous surfaces) for the 120-min contact time, the efficacy was higher at 75% RH than at 40% or 60% RH.

Table 5-5. Summary of Efficacy (Calculated as Mean Log Reduction) of ClO_2 against *B. suis*

		Mean Log Reduction (95% CI) and p-Value* or Mean Log Reduction (# of treated coupons with zero recovery/# of treated coupons) and p-Value† or N/A (# of control coupons with zero recovery/# of control coupons # of treated coupons with zero recovery/# of treated coupons) and p-Value‡		
Material	**% RH**	**30 min**	**60 min**	**120 min**
Aluminum	40	**0.28 (0.19, 0.37) p=0.0002**	**0.32 (0.25, 0.40) p<0.0001**	**1.02 (0.83, 1.20) p<0.0001**
	60	**0.98 (0.85, 1.11) p<0.0001**	**1.02 (0.53, 1.52) p=0.0081**	**3.52 (3.04, 3.99) p<0.0001**
	75	**>5.20 (2/5) p=0.0079**	**>7.50 (5/5) p=0.0079**	**>7.33 (5/5) p=0.0079**
Carpet	40	**>7.06 (4/5) p=0.0079**	**2.79 (2.07, 3.52) p=0.0003**	**>5.22 (5/5) p=0.0079**
	60	**3.85 (3.31, 4.39) p<0.0001**	**>2.09 (3/5) p=0.0079**	**>4.96 (5/5) p=0.0079**
	75	**>6.68 (5/5) p=0.0079**	**>5.59 (5/5) p=0.0079**	**>4.81 (5/5) p=0.0079**
Keyboard	40	0.13 (-0.035, 0.29) p=0.1090	**0.64 (0.42, 0.86) p=0.0002**	**0.72 (0.57, 0.87) p=0.0002**
	60	**0.35 (0.14, 0.56) p=0.0047**	**0.28 (0.22, 0.33) p<0.0001**	**0.95 (0.76, 1.15) p<0.0001**
	75	**2.54 (1.34, 3.75) p=0.0046**	**0.91 (0.71, 1.10) p<0.0001**	**>5.63 (3/5) p=0.0079**
Joint Tape	40	>1.08 (3/5) p=0.3571	**0.70 (0.043, 1.35) p=0.0408**	N/A (5/5 5/5) p=1.0000
	60	**N/A (1/5 5/5) p=0.0476**	**>2.35 (5/5) p=0.0079**	**>2.29 (5/5) p=0.0079**
	75	**>3.78 (5/5) p=0.0079**	N/A (2/5 5/5) p=0.1667	N/A (2/5 5/5) p=0.1667

* Mean log reduction is the mean of the base-10 logarithm of recovered agent from the control coupons minus the mean of the base-10 logarithm of recovered agent from the treated coupons. A 95 % CI for the difference is shown in parentheses. A p-value is provided for the probability that the control and treatment recoveries are the same. The p-value is from the two sample t-test with Satterthwaite's method to allow for potentially different variances in the two groups. p-Values less than 0.05 denote less than 1 in 20 chance that a difference as large as or larger than observed would occur by chance if the control and treatment means were truly identical. Comparisons with p-values less than 0.05 (statistically significant at the 0.05 level) are bolded.

† One or more of the treatment coupons had no recovered agent. The mean log reduction of the form ">X" is calculated as the mean of the base-10 logarithm of recovered agent from the control coupons minus the mean of the base-10 logarithm of recovered agent from the treated coupons except that "zero recovery" coupons have a substituted recovered value of "1" (base-10 log is 0). Since the log becomes an increasingly negative value below 1 and is undefined at 0, this substitution is necessary and results in a lower bound on the mean log difference, as indicated by the ">". The number of "zero recovery" treatment coupons and the total number of treatment coupons is shown in parentheses. The p-value is from the non-parametric Kolmogorov-Smirnov test. p-Values less than 0.05 denote less than 1 in 20 chance that results as different as or more different than observed would occur by chance if the distribution of the control and treatment recoveries were truly identical. Comparisons with p-values less than 0.05 (statistically significant at the 0.05 level) are bolded.

‡ One or more of both the control and the treatment coupons had no recovered agent. In this case, the log reduction is indeterminate and the mean log reduction is identified as "N/A". The number of "zero recovery" control coupons and the total number of control coupons are shown in parentheses followed by the number of "zero recovery" treatment coupons and the total number of treatment coupons. The p-value is from the non-parametric Kolmogorov-Smirnov test. p-Values less than 0.05 denote less than 1 in 20 chance that results as different as or more different than observed would occur by chance if the distribution of the control and treatment recoveries were truly identical. Comparisons with p-values less than 0.05 (statistically significant at the 0.05 level) are bolded.

F. tularensis

Sabre ClO_2 fumigation results for *F. tularensis* are presented in Tables 5-6 and 5-7 and Figures 5-7 and 5-8. Persistence tests reported in Section 4.2.2, as well as positive controls used in these tests, showed that there was a differential loss of viability of *F. tularensis*, depending on the coupon type. Specifically, over the 1hr drying time and multiple contact times, a substantial reduction in viable *F. tularensis* on carpet and joint tape was observed at 75% RH. As described in the Introduction, the calculated log reductions reflect the incremental impact of the fumigation technology. Viable *F. tularensis* was not recovered from any material following the exposure to 50-100 ppmv ClO_2 for a 120-min contact time at 75% RH.

No viable *F. tularensis* was recovered from aluminum and keyboard exposed to 50-100 ppmv ClO_2 (23 ^{o}C) for a contact time of 120-min at 75% RH. For aluminum and keyboard, log reductions generally increased with increasing CTs and log reductions were generally higher at 75% RH than the associated test at 40% RH.

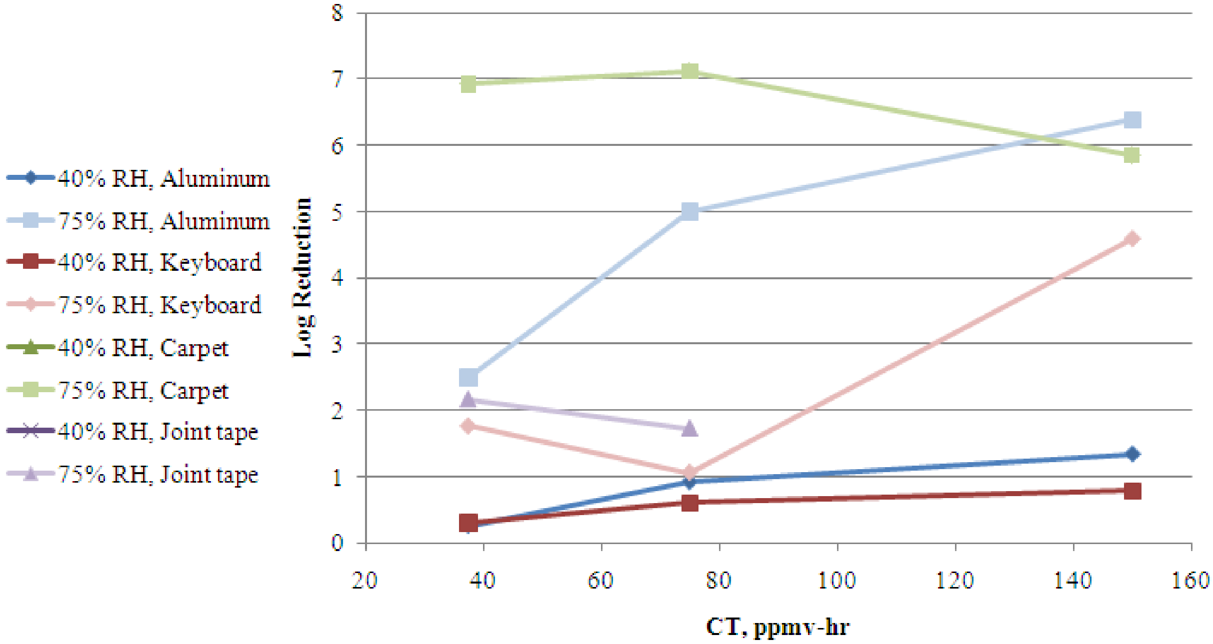
There was a decrease in the recovery of viable *F. tularensis* from carpet and joint tape positive control coupons (not exposed to fumigation) during the period of decontamination. The decline in viable *F. tularensis* from carpet and joint tape at 75% RH was consistent with the >5 log loss of viable *F. tularensis* from carpet and joint tape after 2 hr that was observed in the persistence testing. During fumigation testing with ClO_2, the positive control coupons were allowed to dry for one hr; and an additional period of time (about 30 min) passes before the CT clock starts for fumigation. Thus, after 30-min fumigation, the positive controls may be at a time equivalent to 2 hr in the persistence testing. There was a decrease in the recovery of viable *F. tularensis* from carpet and joint tape positive control coupons (not exposed to fumigation) during the period of decontamination that was consistent with the loss of viable *F. tularensis* from carpet and joint tape in the persistence testing. Because the log reduction reflects only the incremental impact of fumigation (controls for loss of viability without treatment), overall efficacy must be interpreted in the context of the loss of viability with treatment. For example, joint tape exposed to the fumigation treatment for 30 min at 75% RH shows a log reduction of only 2.17. However, only about 10^2 bacteria were recovered from the untreated carpet. (This results in a very low base to which the treated carpet was compared.) Compared to the amount of bacteria spiked onto the coupon (6.77 x 10^7 CFUs), no bacteria recovered after the 30-min treatment (0 CFUs/coupon) represented about a 7.8 log reduction in viable bacteria attributable to the fumigation and the loss of viability from joint tape arising from other (unknown) causes.

Table 5-6. Sabre ClO_2 Fumigation Results for *F. tularensis*

Contact Time	Material	Spike Amount (CFU/coupon)	Mean Recovered F. tularensis (CFU/coupon)*		Mean Log Reduction*
			Positive Control†	Test Coupon‡	
50-100 ppmv ClO_2, 23°C, 40% RH					
0 min	Aluminum	5.17 x 10^7	1.68 ± 0.88 x 10^7	Not applicable	Not applicable
	Keyboard	5.17 x 10^7	2.47 ± 1.25 x 10^6	Not applicable	Not applicable
	Carpet	5.17 x 10^7	3.76 ± 4.80 x 10^6	Not applicable	Not applicable
	Joint tape	5.17 x 10^7	3.07 ± 1.30 x 10^2	Not applicable	Not applicable
30 min	Aluminum	5.17 x 10^7	1.88 ± 0.87 x 10^6	1.35 ± 0.58 x 10^6	0.19 ± 0.23
	Keyboard	5.17 x 10^7	1.87 ± 0.70 x 10^6	9.54 ± 1.58 x 10^5	0.30 ± 0.07
	Carpet	5.17 x 10^7	0.00 ± 0.00	0.00 ± 0.00	Not calculable
	Joint tape	5.17 x 10^7	0.00 ± 0.00	0.00 ± 0.00	Not calculable
60 min	Aluminum	1.05 x $10^{8\#}$	4.28 ± 1.35 x 10^6	6.22 ± 2.95 x 10^5	0.92 ± 0.36
	Keyboard	1.05 x $10^{8\#}$	3.46 ± 0.23 x 10^6	9.49 ± 3.73 x 10^5	0.60 ± 0.23
	Carpet	1.05 x $10^{8\#}$	0.00 ± 0.00	0.00 ± 0.00	Not calculable
	Joint tape	1.05 x $10^{8\#}$	0.00 ± 0.00	0.00 ± 0.00	Not calculable
120 min	Aluminum	1.05 x $10^{8\#}$	3.95 ± 0.78 x 10^6	1.92 ± 0.60 x 10^5	1.34 ± 0.18
	Keyboard	1.05 x $10^{8\#}$	3.15 ± 0.93 x 10^6	5.52 ± 1.75 x 10^5	0.78 ± 0.17
	Carpet	1.05 x $10^{8\#}$	0.00 ± 0.00	0.00 ± 0.00	Not calculable
	Joint tape	1.05 x $10^{8\#}$	0.00 ± 0.00	0.00 ± 0.00	Not calculable
50-100 ppmv ClO_2, 23°C, 75% RH					
0 min	Aluminum	6.77 x 10^7	5.39 ± 1.36 x 10^7	Not applicable	Not applicable
	Keyboard	6.77 x 10^7	4.35 ± 0.78 x 10^6	Not applicable	Not applicable
	Carpet	6.77 x 10^7	2.22 ± 0.28 x 10^8	Not applicable	Not applicable
	Joint tape	6.77 x 10^7	0.00 ± 0.00	Not applicable	Not applicable
30 min	Aluminum	6.77 x $10^{7\,\S}$	1.93 ± 1.39 x 10^7	1.28 ± 1.69 x 10^5	2.50 ± 0.60
	Keyboard	6.77 x $10^{7\,\S}$	3.11 ± 0.26 x 10^6	5.70 ± 1.88 x 10^4	1.76 ± 0.17
	Carpet	6.77 x $10^{7\,\S}$	8.36 ± 4.48 x 10^6	0.00 ± 0.00	6.92 ± 0.00
	Joint tape	6.77 x $10^{7\,\S}$	1.47 ± 2.22 x 10^2	0.00 ± 0.00	2.17 ± 0.00
60 min	Aluminum	1.15 x $10^{8\#}$	1.80 ± 1.43 x 10^7	4.31 ± 5.08 x 10^3	5.00 ± 2.08
	Keyboard	1.15 x $10^{8\#}$	9.45 ± 2.67 x 10^5	9.03 ± 4.35 x 10^4	1.06 ± 0.21
	Carpet	1.15 x $10^{8\#}$	1.30 ± 0.89 x 10^7	0.00 ± 0.00	7.12 ± 0.00
	Joint tape	1.15 x $10^{8\#}$	5.32 ± 10.2 x 10^1	0.00 ± 0.00	1.73 ± 0.00
120 min	Aluminum	6.77 x 10^7	2.39 ± 1.41 x 10^6	0.00 ± 0.00	6.38 ± 0.00
	Keyboard	6.77 x 10^7	3.86 ± 0.82 x 10^4	0.00 ± 0.00	4.59 ± 0.00
	Carpet	6.77 x 10^7	7.01 ± 9.14 x 10^5	0.00 ± 0.00	5.85 ± 0.00
	Joint tape	6.77 x 10^7	0.00 ± 0.00	0.00 ± 0.00	Not calculable

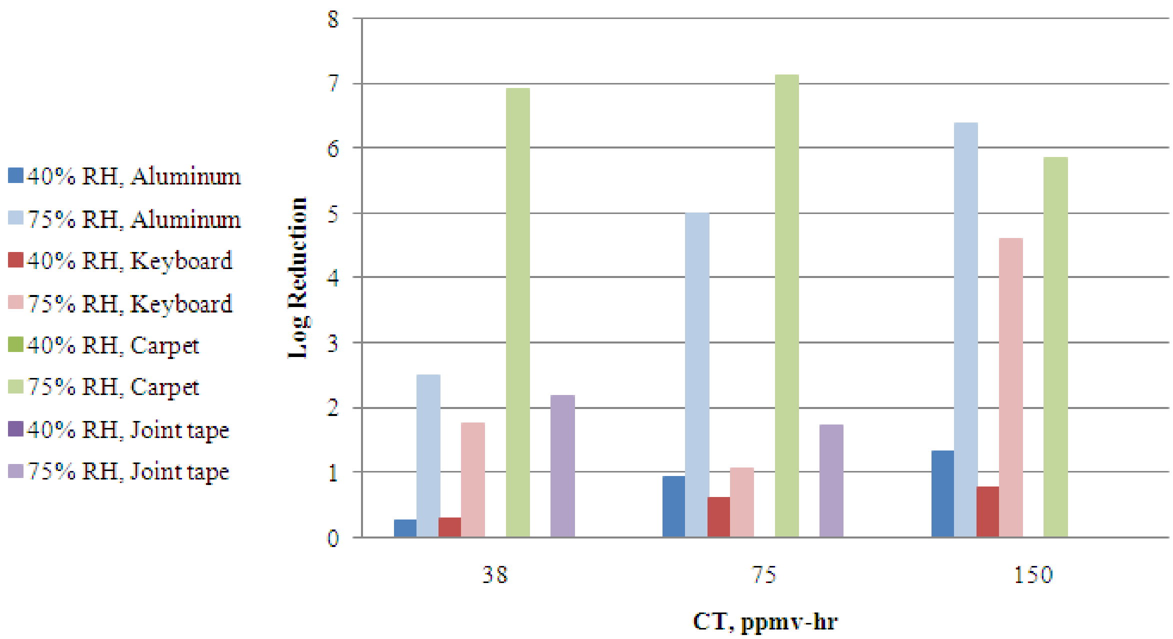
* Data are expressed as mean ± standard deviation of five replicates.
† Positive control coupons were spiked but not exposed to the fumigant.
‡ Test coupons were spiked and exposed to the fumigant for the contact time.
§ The spike amount for the associated positive control was 1.15 x 10^8 CFUs/coupon.
# Exceeds target application of 1.0 x 10^8 CFUs/coupon.

Figure 5-7. Sabre Fumigation Results for *F. tularensis* at 50-100 ppmv ClO_2 and 23 °C, Line Chart.



* Log reductions not calculated for some or all CTs as *F. tularensis* was not recovered from either the test or associated control coupons.

Figure 5-8. Sabre Fumigation Results for *F. tularensis* at 50-100 ppmv ClO_2 and 23 °C, Column Chart.



* Log reductions not calculated for some or all CTs as *F. tularensis* was not recovered from either the test or associated control coupons.

Table 5-7 provides a summary of ClO_2 decontamination efficacy against *F. tularensis*, calculated as the difference in the mean log of viable bacteria recovered from positive control coupons and the mean log of viable bacteria recovered from coupons after fumigation for a given contact time. The elapsed time from spiking to recovery was the same for the positive control coupons and test coupons to control for history. The 95% CI and p-value are also shown. At the 120-min contact time (75% RH), no viable bacteria were recovered from any test coupons.

Because of the temporal loss of viable bacteria at the 120-min contact time statistically significant incremental reduction in viable bacteria arising from the fumigation treatment was demonstrated only for aluminum and keyboard. However, for the carpet and joint tape, a 7.8 log reduction in viable bacteria was attributable to the combined effects of fumigation and the loss of viability over time arising from other (unknown) causes.

Table 5-7. Summary of Efficacy (Calculated as Mean Log Reduction) of ClO_2 against *F. tularensis*

		Mean Log Reduction (95% CI) and p-Value* or Mean Log Reduction (# of treated coupons with zero recovery/# of treated coupons) and p-Value† or N/A (# of control coupons with zero recovery/# of control coupons # of treated coupons with zero recovery/# of treated coupons) and p-Value‡		
Material	**% RH**	**30 min**	**60 min**	**120 min**
Aluminum	40	0.14 (-0.19, 0.48) p=0.3589	**0.90 (0.51, 1.30) p=0.0031**	**1.33 (1.13, 1.53) p<0.0001**
	75	**2.16 (1.44, 2.88) p=0.0003** §	**>4.80 (2/5) p=0.0079**	**>6.32 (5/5) p=0.0079**
Carpet	40	N/A (5/5 5/5) p=1.0000	N/A (5/5 5/5) p=1.0000	N/A (5/5 5/5) p=1.0000
	75	**>6.64 (5/5) p=0.0079** §	**>6.76 (5/5) p=0.0079**	N/A (2/5 5/5) p=0.1667
Keyboard	40	**0.27 (0.097, 0.45) p=0.0132**	**0.60 (0.36, 0.84) p=0.0042**	**0.76 (0.53, 0.99) p<0.0001**
	75	**1.53 (1.35, 1.71) p<0.0001** §	**1.05 (0.80, 1.29) p<0.0001**	**>4.58 (5/5) p=0.0079**
Joint Tape	40	N/A (5/5 5/5) p=1.0000	N/A (5/5 5/5) p=1.0000	N/A (5/5 5/5) p=1.0000
	75	N/A (3/5 5/5) p=0.1667 §	N/A (3/5 5/5) p=0.4444	N/A (5/5 5/5) p=1.0000

* Mean log reduction is the mean of the base-10 logarithm of recovered agent from the control coupons minus the mean of the base-10 logarithm of recovered agent from the treated coupons. A 95 % CI for the difference is shown in parentheses. A p-value is provided for the probability that the control and treatment recoveries are the same. The p-value is from the two sample t-test with Satterthwaite's method to allow for potentially different variances in the two groups. p-Values less than 0.05 denote less than 1 in 20 chance that a difference as large as or larger than observed would occur by chance if the control and treatment means were truly identical. Comparisons with p-values less than 0.05 (statistically significant) are bolded.

† One or more of the treatment coupons had no recovered agent. The mean log reduction of the form ">X" is calculated as the mean of the base-10 logarithm of recovered agent from the control coupons minus the mean of the base-10 logarithm of recovered agent from the treated coupons except that "zero recovery" coupons have a substituted recovered value of "1" (base-10 log is 0). Since the log becomes an increasingly negative value below 1 and is undefined at 0, this substitution is necessary and results in a lower bound on the mean log difference, as indicated by the ">". The number of "zero recovery" treatment coupons and the total number of treatment coupons are shown in parentheses. The p-value is from the non-parametric Kolmogorov-Smirnov test. p-Values less than 0.05 denote less than 1 in 20 chance that results as different as or more different than observed would occur by chance if the distribution of the control and treatment recoveries were truly identical. Comparisons with p-values less than 0.05 (statistically significant at the 0.05 level) are bolded.

‡ One or more of both the control and the treatment coupons had no recovered agent. In this case, the log reduction is indeterminate and the mean log reduction is identified as "N/A". The number of "zero recovery" control coupons and the total number of control coupons are shown in parentheses followed by the number of "zero recovery" treatment coupons and the total number of treatment coupons. The p-value is from the non-parametric Kolmogorov-Smirnov test. p-Values less than 0.05 denote less than 1 in 20 chance that results as different as or more different than observed would occur by chance if the distribution of the control and treatment recoveries were truly identical. Comparisons with p-values less than 0.05 (statistically significant at the 0.05 level) are bolded.

§ The inoculum concentrations for control and treatment coupons were different. Where reported, the mean log reduction is calculated as a relative difference in log reduction for each of controls and treated coupons relative to their respective inoculum concentrations.

Vaccinia Virus

Sabre ClO_2 fumigation of vaccinia virus was at 50-100 ppmv ClO_2 (23 °C). Three RH conditions, 40%, 60%, and 75%, were evaluated. Sabre ClO_2 fumigation results for vaccinia virus are presented in Tables 5-8 and 5-9, and Figures 5-9 and 5-10. No vaccinia virus was recovered from aluminum, carpet, or joint tape after 30-min fumigation at 75% RH; viable vaccinia virus was recovered from keyboard after 120-min fumigation at 75% RH. The greater resistance to decontamination may be attributable to the difference in application on the keyboard due to its small surface: a single 100 µL droplet was used rather than 10 x 10 µL droplets on the other coupon materials. Vaccinia virus was not recovered from carpet or joint tape (log reductions ≥ 6) following any Sabre ClO_2 fumigation treatment at 60% and 75% RH, but was recovered intermittently from aluminum (i.e., from the 120-min contact time at 60% RH) and was generally recovered from keyboard under all tested conditions. Log reductions attributable to the decontamination treatment were generally higher at 60% and 75% RH than at 40% RH.

As shown in Table 5-8 and Figure 5-9, at 40% RH the efficacy against vaccinia appears to decline with longer contact time. High variability in efficacy results was often observed in the transition from low efficacy to high efficacy. At 40% RH, both the 60-min and 120-min contact times are in the high variability transition range. There appears to be an unknown factor that differs between the 60-min test and the 120-min test that causes a decline in the number of viable vaccinia recovered at the longer contact time. (The negative slopes for aluminum and carpet at 75% RH reflect only the difference in the spike amount between tests; no viable virus was recovered from the test coupons at either contact time.)

Decontamination testing was performed at 40% and 75% RH before the testing was performed at 60% RH. Because vaccinia virus had survived at both 40% and 75% RH on keyboard after a 120-min contact time, the observation that no virus was recovered from keyboard after a 120-min contact time at 60% RH was an unexpected result. The 120-min contact time at 60% RH was repeated. The result of the second test (6.84 x 10^4 virus recovered from keyboard) was consistent with previous test results. No reason was identified to explain the anomaly. In parallel EPA testing (report to be completed in May, 2010), a CT of 125 ppm-hr of ClO_2 (80% - 83% RH) resulted in no vaccinia virus being recovered from any coupon of any of the seven materials tested (glass, painted concrete, galvanized metal, decorative laminate, cellulose insulation, particle board, and industrial carpet). Because the application of vaccinia onto keyboard was in a single 100 µL droplet, rather than the 10 x 10 µL droplets applied to the other coupons, the thickness of the dried droplet may provide a protection for interior virus from decontamination resulting in lower efficacy than was observed with the other materials.

Table 5-8. Sabre ClO_2 Fumigation Results for Vaccinia Virus

Contact Time	Material	Spike Amount (PFU/coupon)	Mean Recovered Vaccinia Virus (PFU/coupon)*		Mean Log Reduction*
			Positive Control†	Test Coupon‡	
50-100 ppmv ClO_2, 23°C, 40% RH					
0 min	Aluminum	1.98×10^{7}	$4.68 \pm 1.68 \times 10^{7\S}$	Not applicable	Not applicable
	Keyboard	1.98×10^{7}	$5.77 \pm 2.70 \times 10^{7\S}$	Not applicable	Not applicable
	Carpet	1.98×10^{7}	$1.13 \pm 0.50 \times 10^{7\S}$	Not applicable	Not applicable
	Joint tape	1.98×10^{7}	$6.78 \pm 1.50 \times 10^{6\S}$	Not applicable	Not applicable
60 min	Aluminum	4.82×10^{7}	$1.06 \pm 0.90 \times 10^{8\S}$	$4.68 \pm 0.20 \times 10^{6}$	1.35 ± 0.02
	Keyboard	4.82×10^{7}	$8.03 \pm 4.91 \times 10^{7\S}$	$5.90 \pm 0.84 \times 10^{6}$	1.14 ± 0.06
	Carpet	4.82×10^{7}	$5.92 \pm 2.12 \times 10^{6\S}$	$1.13 \pm 0.99 \times 10^{0}$	6.70 ± 0.22
	Joint tape	4.82×10^{7}	$1.57 \pm 1.19 \times 10^{5\S}$	$6.68 \pm 14.9 \times 10^{-2}$	5.29 ± 0.21
120 min	Aluminum	1.98×10^{7}	$4.64 \pm 2.99 \times 10^{7\S}$	$4.48 \pm 1.46 \times 10^{6}$	1.03 ± 0.15
	Keyboard	1.98×10^{7}	$3.75 \pm 1.18 \times 10^{7\S}$	$9.95 \pm 3.33 \times 10^{6}$	0.60 ± 0.17
	Carpet	1.98×10^{7}	$7.49 \pm 3.05 \times 10^{6\S}$	$2.68 \pm 2.10 \times 10^{4}$	2.57 ± 0.40
	Joint tape	1.98×10^{7}	$2.29 \pm 0.62 \times 10^{6\S}$	$1.27 \pm 0.96 \times 10^{4}$	2.65 ± 1.04
50-100 ppmv ClO_2, 23°C, 60% RH					
0 min	Aluminum	2.60×10^{8}	$5.34 \pm 1.58 \times 10^{7\S}$	Not applicable	Not applicable
	Keyboard	2.60×10^{8}	$6.95 \pm 5.19 \times 10^{7\S}$	Not applicable	Not applicable
	Carpet	2.60×10^{8}	$2.01 \pm 1.80 \times 10^{7\S}$	Not applicable	Not applicable
	Joint tape	2.60×10^{8}	$3.01 \pm 4.77 \times 10^{7\S}$	Not applicable	Not applicable
120 min (Initial)	Aluminum	8.17×10^{8}	$1.83 \pm 0.23 \times 10^{8\S}$	0.00 ± 0.00	8.26 ± 0.00
	Keyboard	8.17×10^{8}	$1.44 \pm 0.71 \times 10^{8\S}$	0.00 ± 0.00	8.16 ± 0.00
	Carpet	8.17×10^{8}	$3.16 \pm 5.00 \times 10^{7\S}$	0.00 ± 0.00	7.50 ± 0.00
	Joint tape	8.17×10^{8}	$2.29 \pm 2.71 \times 10^{6\S}$	0.00 ± 0.00	6.36 ± 0.00
120 min (Repeat)	Aluminum	2.60×10^{8}	$6.23 \pm 4.83 \times 10^{7\S}$	$3.55 \pm 0.54 \times 10^{4}$	3.25 ± 0.07
	Keyboard	2.60×10^{8}	$2.83 \pm 2.05 \times 10^{7\S}$	$6.84 \pm 2.14 \times 10^{4}$	2.63 ± 0.14
	Carpet	2.60×10^{8}	$3.48 \pm 5.14 \times 10^{7\S}$	0.00 ± 0.00	7.54 ± 0.00
	Joint tape	2.60×10^{8}	$9.96 \pm 2.95 \times 10^{5\S}$	0.00 ± 0.00	6.00 ± 0.00
50-100 ppmv ClO_2, 23°C, 75% RH					
0 min	Aluminum	6.09×10^{8}	$8.54 \pm 3.60 \times 10^{7\S}$	Not applicable	Not applicable
	Keyboard	6.09×10^{8}	$1.04 \pm 0.78 \times 10^{8}$	Not applicable	Not applicable
	Carpet	6.09×10^{8}	$2.70 \pm 1.20 \times 10^{7\S}$	Not applicable	Not applicable
	Joint tape	6.09×10^{8}	$5.86 \pm 2.73 \times 10^{6\S}$	Not applicable	Not applicable
30 min	Aluminum	6.09×10^{8}	$1.20 \pm 0.79 \times 10^{8\S}$	0.00 ± 0.00	8.08 ± 0.00
	Keyboard	6.09×10^{8}	$9.51 \pm 5.18 \times 10^{7\S}$	$1.08 \pm 0.35 \times 10^{6}$	1.96 ± 0.15
	Carpet	6.09×10^{8}	$4.47 \pm 4.87 \times 10^{7\S}$	0.00 ± 0.00	7.65 ± 0.00
	Joint tape	6.09×10^{8}	$2.77 \pm 4.05 \times 10^{6\S}$	0.00 ± 0.00	6.44 ± 0.00
60 min	Aluminum	1.20×10^{8}	$3.07 \pm 0.83 \times 10^{7\S}$	0.00 ± 0.00	7.49 ± 0.00
	Keyboard	1.20×10^{8}	$4.06 \pm 0.94 \times 10^{7}$	$1.33 \pm 0.35 \times 10^{5}$	2.50 ± 0.11
	Carpet	1.20×10^{8}	$9.81 \pm 2.86 \times 10^{6\S}$	0.00 ± 0.00	6.99 ± 0.00
	Joint tape	1.20×10^{8}	$7.67 \pm 1.93 \times 10^{6\S}$	0.00 ± 0.00	6.89 ± 0.00
120 min	Keyboard	3.97×10^{8}	$1.87 \pm 0.77 \times 10^{8\S}$	$4.13 \pm 1.84 \times 10^{4}$	3.69 ± 0.21

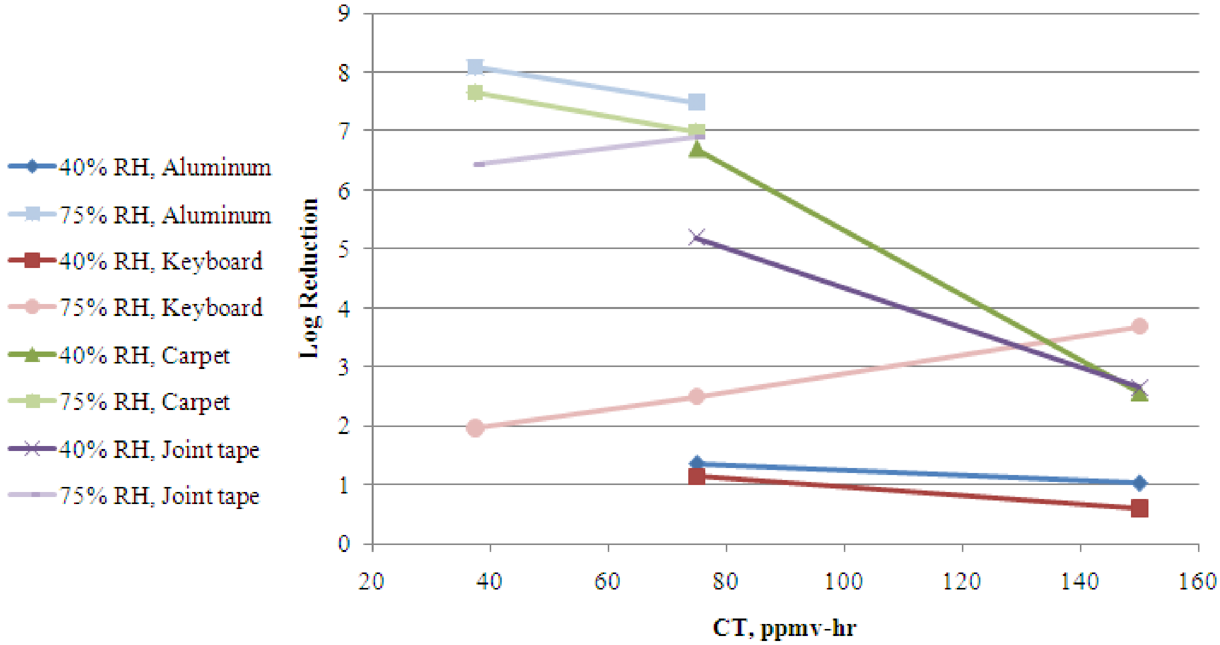
* Data are expressed as mean ± standard deviation of five replicates.

† Positive control coupons were spiked but not exposed to the fumigant.

‡ Test coupons were spiked and exposed to the fumigant for the contact time.

§ Exceeded the positive control CV target of ≤25%.

Figure 5-9. Sabre Fumigation Results for Vaccinia Virus at 50-100 ppmv ClO, and 23 °C, Line Chart.



Note: Log reductions associated with 60% RH are not shown as data were only generated at 150 CT, ppmv-hr.

Figure 5-10. Sabre Fumigation Results for Vaccinia Virus at 50-100 ppmv ClO_2 and 23 °C, Column Chart ("a" Indicates Initial Test; "b" Indicates Repeat Test).

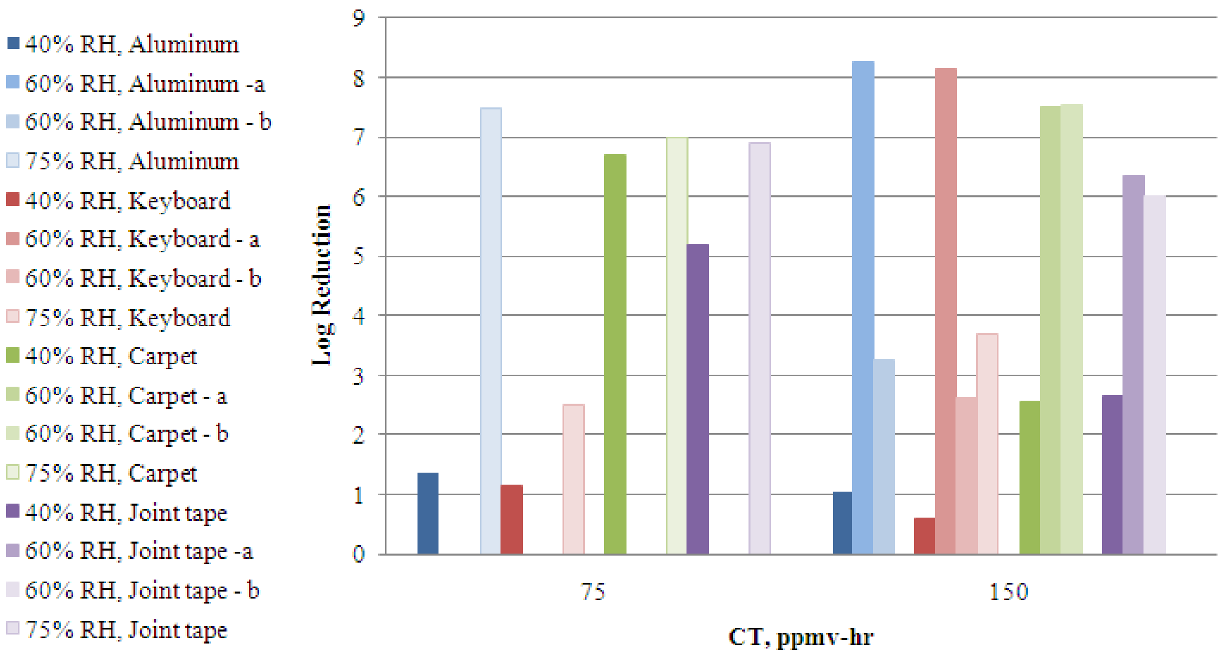


Table 5-9 provides a summary of ClO_2 decontamination efficacy against vaccinia virus, calculated as the difference in the mean log of viable (plaque-forming) virus recovered from positive control coupons and the mean log of viable virus recovered from coupons after fumigation for a given contact time. The elapsed time from spiking to recovery was the same for the positive control coupons and test coupons to control for history. The 95% CI and p-value are also shown. At the 30-min and 60-min contact times (75% RH), no viable virus was recovered from any aluminum, carpet, or joint tape test coupons. Viable virus was recovered from keyboard coupons at the 120-min contact time (75% RH). Efficacy was higher at 75% RH than at 40% RH at all contact times tested for aluminum, carpet, and joint tape test coupons.

Table 5-9. Summary of Efficacy (Calculated as Mean Log Reduction) of ClO_2 against Vaccinia Virus

	Mean Log Reduction (95% CI) and p-Value* or Mean Log Reduction (# of treated coupons with zero recovery/# of treated coupons) and p-Value†			
Material	**% RH**	**30 min**	**60 min**	**120 min**
Aluminum	40		**1.21 (0.80, 1.62) p=0.0024**	**0.97 (0.66, 1.27) p=0.0003**
	60			**>5.70 (5/10) p<0.0001**
	75	**>7.98 (5/5) p=0.0079**	**>7.48 (5/5) p=0.0079**	
Carpet	40		**>6.67 (1/5) p=0.0079**	**2.55 (2.11, 2.99) p<0.0001**
	60			**>7.10 (10/10) p<0.0001**
	75	**>7.47 (5/5) p=0.0079**	**>6.98 (5/5) p=0.0079**	
Keyboard	40		**1.07 (0.80, 1.35) p=0.0006**	**0.58 (0.37, 0.80) p=0.0003**
	60			**>5.29 (5/10) p<0.0001**
	75	**1.91 (1.61, 2.21) p<0.0001**	**2.49 (2.33, 2.64) p<0.0001**	**3.64 (3.30, 3.99) p<0.0001**
Joint Tape	40		**>4.67 (4/5) p=0.0079**	**2.64 (1.56, 3.72) p=0.0045**
	60			**>5.93 (10/10) p<0.0001**
	75	**>6.06 (5/5) p=0.0079**	**>6.87 (5/5) p=0.0079**	

* Mean log reduction is the mean of the base-10 logarithm of recovered agent from the control coupons minus the mean of the base-10 logarithm of recovered agent from the treated coupons. A 95 % CI for the difference is shown in parentheses. A p-value is provided for the probability that the control and treatment recoveries are the same. The p-value is from the two sample t-test with Satterthwaite's method to allow for potentially different variances in the two groups. p-Values less than 0.05 denote less than 1 in 20 chance that a difference as large as or larger than observed would occur by chance if the control and treatment means were truly identical. Comparisons with p-values less than 0.05 (statistically significant at the 0.05 level) are bolded.

† One or more of the treatment coupons had no recovered agent. The mean log reduction of the form ">X" is calculated as the mean of the base-10 logarithm of recovered agent from the control coupons minus the mean of the base-10 logarithm of recovered agent from the treated coupons except that "zero recovery" coupons have a substituted recovered value of "1" (base-10 log is 0). Since the log becomes an increasingly negative value below 1 and is undefined at 0, this substitution is necessary and results in a lower bound on the mean log difference, as indicated by the ">". The number of "zero recovery" treatment coupons and the total number of treatment coupons is shown in parentheses. The p-value is from the non-parametric Kolmogorov-Smirnov test. p-Values less than 0.05 denote less than 1 in 20 chance results as different or more different than observed would occur by chance if the distribution of the control and treatment recoveries were truly identical. Comparisons with p-values less than 0.05 (statistically significant at the 0.05 level) are bolded.

Y. pestis

Sabre ClO_2 fumigation results for *Y. pestis* are presented in Tables 5-10 and 5-11. Persistence tests reported in Section 4.2.4, as well as positive controls used in these tests, showed that there was a differential loss of viability of *Y. pestis*, depending on the coupon type. However, persistence was higher during the persistence testing than was observed from positive controls. Both the persistence testing and the positive controls for the various fumigation tests demonstrate that viable *Y. pestis* spores are rapidly lost from the various material coupons under the conditions tested. As described in the Introduction, the calculated log reductions reflect the incremental impact of the fumigation technology. *Y. pestis* was not recovered following any Sabre ClO_2 fumigation treatment of 30 min or longer. Variable amounts of *Y. pestis* were recovered from positive control coupons. *Y. pestis* was often not recovered from the associated positive controls; mean log reductions could not be calculated for the Sabre ClO_2 fumigation.

Because the log reduction reflects only the incremental impact of fumigation (controls for loss of viability without treatment), overall efficacy must be interpreted in the context of the loss of viability with treatment. Compared to the amount of bacteria spiked onto the coupon (about 10^7 CFUs/coupon), no bacteria were recovered after the 30-min treatment (0 CFUs/coupon) at either the 40% RH or 75% RH condition. The loss of viable bacteria represents >7 log reduction in viable bacteria attributable to the fumigation and the loss of viability over time from other (unknown) causes. The causes of variability in the rates of decline in recoverable *Y. pestis* bacteria from positive control coupons and in persistence testing (no fumigation) are unknown.

Table 5-10. Sabre ClO_2 Fumigation Results for *Y. pestis*

Contact Time	Material	Spike Amount (CFU/coupon)	Mean Recovered *Y. pestis* (CFU/coupon)*		Mean Log Reduction*
			Positive Control†	Test Coupon‡	
50-100 ppmv ClO_2, 23°C, 40% RH					
0 min	Aluminum	2.30 x 10^7	9.54 ± 5.52 x 10^3	Not applicable	Not applicable
	Keyboard	2.30 x 10^7	6.16 ± 6.09 x 10^3	Not applicable	Not applicable
	Carpet	2.30 x 10^7	5.04 ± 2.64 x 10^6	Not applicable	Not applicable
	Joint tape	2.30 x 10^7	0.00 ± 0.00	Not applicable	Not applicable
30 min	Aluminum	1.56 x 10^7	0.00 ± 0.00	0.00 ± 0.00	Not calculable
	Keyboard	1.56 x 10^7	4.00 ± 7.24 x 10^1	0.00 ± 0.00	1.60 ± 0.00
	Carpet	1.56 x 10^7	1.05 ± 1.10 x 10^6	0.00 ± 0.00	6.02 ± 0.00
	Joint tape	1.56 x 10^7	0.00 ± 0.00	0.00 ± 0.00	Not calculable
60 min	Aluminum	2.30 x 10^7	1.33 ± 1.23 x 10^2	0.00 ± 0.00	2.13 ± 0.00
	Keyboard	2.30 x 10^7	2.59 ± 3.42 x 10^3	0.00 ± 0.00	3.41 ± 0.00
	Carpet	2.30 x 10^7	0.00 ± 0.00	0.00 ± 0.00	Not calculable
	Joint tape	2.30 x 10^7	0.00 ± 0.00	0.00 ± 0.00	Not calculable
120 min	Aluminum	1.56 x 10^7	0.00 ± 0.00	0.00 ± 0.00	Not calculable
	Keyboard	1.56 x 10^7	0.00 ± 0.00	0.00 ± 0.00	Not calculable
	Carpet	1.56 x 10^7	0.00 ± 0.00	0.00 ± 0.00	Not calculable
	Joint tape	1.56 x 10^7	0.00 ± 0.00	0.00 ± 0.00	Not calculable
50-100 ppmv ClO_2, 23°C, 75% RH					
0 min	Aluminum	3.03 x 10^7	1.23 ± 0.79 x 10^4	Not applicable	Not applicable
	Keyboard	3.03 x 10^7	1.18 ± 0.45 x 10^4	Not applicable	Not applicable
	Carpet	3.03 x 10^7	8.69 ± 2.60 x 10^6	Not applicable	Not applicable
	Joint tape	3.03 x 10^7	7.32 ± 10.6 x 10^1	Not applicable	Not applicable
30 min	Aluminum	1.77 x 10^7	0.00 ± 0.00	0.00 ± 0.00	Not calculable
	Keyboard	1.77 x 10^7	0.00 ± 0.00	0.00 ± 0.00	Not calculable
	Carpet	1.77 x 10^7	2.42 ± 2.58 x 10^6	0.00 ± 0.00	6.38 ± 0.00
	Joint tape	1.77 x 10^7	0.00 ± 0.00	0.00 ± 0.00	Not calculable
60 min	Aluminum	3.03 x 10^7	1.53 ± 1.37 x 10^2	0.00 ± 0.00	2.19 ± 0.00
	Keyboard	3.03 x 10^7	1.05 ± 1.12 x 10^3	0.00 ± 0.00	3.02 ± 0.00
	Carpet	3.03 x 10^7	5.02 ± 7.23 x 10^4	0.00 ± 0.00	4.70 ± 0.00
	Joint tape	3.03 x 10^7	0.00 ± 0.00	0.00 ± 0.00	Not calculable
120 min	Aluminum	1.77 x 10^7	0.00 ± 0.00	0.00 ± 0.00	Not calculable
	Keyboard	1.77 x 10^7	0.00 ± 0.00	0.00 ± 0.00	Not calculable
	Carpet	1.77 x 10^7	4.57 ± 9.75 x 10^4	0.00 ± 0.00	4.66 ± 0.00
	Joint tape	1.77 x 10^7	0.00 ± 0.00	0.00 ± 0.00	Not calculable

* Data are expressed as mean ± standard deviation of five replicates.
† Positive control coupons were spiked but not exposed to the fumigant.
‡ Test coupons were spiked and exposed to the fumigant for the contact time.

Summary Statistics for Sabre ClO_2 Decontamination

Table 5-11 provides a summary of ClO_2 decontamination efficacy against *Y. pestis,* calculated as the difference in the mean log of viable bacteria recovered from positive control coupons and the mean log of viable bacteria recovered from coupons after fumigation for a given contact time. The elapsed time from spiking to recovery was the same for the positive control coupons and test coupons to control for history. The 95% CI and p-value are also shown. At the 120-min contact time (75% RH), no viable *Y. pestis* was recovered from any aluminum, keyboard, carpet, or joint tape test coupons. Because of the loss of viable *Y. pestis* from positive control coupons due to unknown, time dependent causes, the log reductions attributable only to the fumigation effect could not be determined; the combined effects represent >7 log reduction in viable bacteria attributable to the fumigation and the loss of viability over time from other (unknown) causes.

Surface Damage

The physical effect of the Sabre ClO_2 fumigation on the materials was evaluated qualitatively. The appearance of the decontaminated coupons was visually inspected for any obvious changes in the color, reflectivity, and apparent roughness of the material surfaces. These comparisons were performed for each material, before extraction of the decontaminated test coupons. No differences were observed between control and fumigated coupons for any material except that at high ClO_2 for prolonged contact times some darkening of the aluminum was observed.

Table 5-11. Summary of Efficacy (Calculated as Mean Log Reduction) of ClO_2 against *Y. pestis*

	Mean Log Reduction (95% CI) and p-Value* or Mean Log Reduction (# of treated coupons with zero recovery/# of treated coupons) and p-Value† or N/A (# of control coupons with zero recovery/# of control coupons # of treated coupons with zero recovery/# of treated coupons) and p-Value‡			
Material	**% RH**	**30 min**	**60 min**	**120 min**
Aluminum	40	N/A (5/5 5/5) p=1.0000	**>1.94 (5/5) p=0.0079**	N/A (5/5 5/5) p=1.0000
	75	N/A (5/5 5/5) p=1.0000	**>2.02 (5/5) p=0.0079**	N/A (5/5 5/5) p=1.0000
Carpet	40	**>5.79 (5/5) p=0.0079**	N/A (5/5 5/5) p=1.0000	N/A (5/5 5/5) p=1.0000
	75	**>5.81 (5/5) p=0.0079**	**>3.81 (5/5) p=0.0079**	N/A (3/5 5/5) p=0.4444
Keyboard	40	0.75 (3/5 5/5) p=0.4444	**>3.18 (5/5) p=0.0079**	N/A (5/5 5/5) p=1.0000
	75	N/A (5/5 5/5) p=1.0000	**>2.80 (5/5) p=0.0079**	N/A (5/5 5/5) p=1.0000
Joint Tape	40	N/A (5/5 5/5) p=1.0000	N/A (5/5 5/5) p=1.0000	N/A (5/5 5/5) p=1.0000
	75	N/A (5/5 5/5) p=1.0000	N/A (5/5 5/5) p=1.0000	N/A (5/5 5/5) p=1.0000

* Mean log reduction is the mean of the base-10 logarithm of recovered agent from the control coupons minus the mean of the base-10 logarithm of recovered agent from the treated coupons. A 95% CI for the difference is shown in parentheses. A p-value is provided for the probability that the control and treatment recoveries are the same. The p-value is from the two sample t-test with Satterthwaite's method to allow for potentially different variances in the two groups. p-Values less than 0.05 denote less than 1 in 20 chance that a difference as large as or larger than observed would occur by chance if the control and treatment means were truly identical. Comparisons with p-values less than 0.05 (statistically significant at the 0.05 level) are bolded.

† One or more of the treatment coupons had no recovered agent. The mean log reduction of the form ">X" is calculated as the mean of the base-10 logarithm of recovered agent from the control coupons minus the mean of the base-10 logarithm of recovered agent from the treated coupons except that "zero recovery" coupons have a substituted recovered value of "1" (base-10 log is 0). Since the log becomes an increasingly negative value below 1 and is undefined at 0, this substitution is necessary and results in a lower bound on the mean log difference, as indicated by the ">". The number of "zero recovery" treatment coupons and the total number of treatment coupons are shown in parentheses. The p-value is from the non-parametric Kolmogorov-Smirnov test. p-Values less than 0.05 denote less than 1 in 20 chance that results as different as or more different than observed would occur by chance if the distribution of the control and treatment recoveries were truly identical. Comparisons with p-values less than 0.05 (statistically significant at the 0.05 level) are bolded.

‡ One or more of both the control and the treatment coupons had no recovered agent. In this case, the log reduction is indeterminate and the mean log reduction is identified as "N/A". The number of "zero recovery" control coupons and the total number of control coupons is shown in parentheses followed by the number of "zero recovery" treatment coupons and the total number of treatment coupons. The p-value is from the non-parametric Kolmogorov-Smirnov test. p-Values less than 0.05 denote less than 1 in 20 chance that results as different as or more different than observed would occur by chance if the distribution of the control and treatment recoveries were truly identical. Comparisons with p-values less than 0.05 (statistically significant at the 0.05 level) are bolded.

5.2 HP Fumigation (BIOQUELL Clarus C)

5.2.1 Description of BIOQUELL Clarus C HP Technology

The following is a description of the BIOQUELL Clarus C unit, based on information provided by the vendor. The information provided below was not verified in this test.

The BIOQUELL Clarus C unit is a hydrogen peroxide gas generator (Figure 5-11) that uses a dual circuit system. The first circuit provides high-efficiency particulate air (HEPA) filtration, dehumidification, and hydrogen peroxide removal from the air stream via catalytic conversion. The second circuit delivers high-concentration HP and water vapors. During gassing, the BIOQUELL Clarus C unit recirculates the vapors through the second circuit, constantly increasing the concentration of HP and water vapor within the chamber or area intended for decontamination. This recirculation and vapor injection continues until the chamber reaches saturation, and the process of microcondensation begins. In microcondensation, a microscopic film of aqueous hydrogen peroxide solution is deposited on all surfaces. Once the gassing phase has been completed, the BIOQUELL Clarus C unit returns to the first circuit and brings the chamber to a safe condition by catalytically converting the hydrogen peroxide to water (humidity) and oxygen. Excess humidity is removed via the refrigerant-based dehumidification system of the BIOQUELL Clarus C unit. To ensure that all essential data are captured, the BIOQUELL Clarus C unit prints out all critical parameters recorded throughout the cycle. The BIOQUELL Clarus C unit has a personal computer connection for more in-depth cycle analysis.

The BIOQUELL Clarus C unit was designed to decontaminate enclosures of up to 200 cubic meters. The unit weighs 128 kilograms and is 68 cm wide by 90 cm in depth by 106 cm in height. The dehumidification system is designed to run continuously. Because there was no need for dehumidification regeneration down-time, the BIOQUELL Clarus C unit can operate continuously, if required, from a normal (120 volts alternating current) domestic power supply. The BIOQUELL Clarus C unit is controlled by a Siemens programmable logic controller, which is complemented by optional sensors (including a microcondensation sensor), allowing repeatable validated decontamination cycles.

For this verification test, the BIOQUELL Clarus C unit was attached to a test chamber that was a BSC III, approximately 1275 L. The BIOQUELL Clarus C unit and the glove box were connected by flexible supply and delivery gassing hoses that contained in-line HEPA filtration. A hydrogen peroxide sensor, HP sensor, and pressure sensing tube were connected to the inside of the test chamber and data were transmitted through the test chamber wall to the BIOQUELL Clarus C unit.

The vendor provided on-site support for the installation of the Clarus C unit and cycle development before testing was begun. Testing was performed by Battelle personnel only. Early tests were observed by a vendor representative.

Figure 5-11. BIOQUELL Clarus C HP Vapor Generator.[14]

HP measurements were made inside the test chamber using an Analytical Technology Model B12 HP gas sensor. In order to detect sudden degradation in the sensor performance, the gas sensor measurements were compared periodically to a titration measurement of air samples drawn through a sampling train of two impingers, each containing 20 mL of 5% sulfuric acid (H_2SO_4). The 40 mL of solution from the impingers was added to 150 mL of deionized water for a total of 190 mL of solution. The solution was titrated with 0.1N or 0.02N potassium permanganate ($KMnO_4$). The total equation can be expressed as: 5 H_2O_2 (hydrogen peroxide) + 2 $KMnO_4$ + 4 $H_2SO_4 \rightarrow$ 2 $KHSO_4$ (potassium sulfate) + 2 $MnSO_4$ (manganese sulfate) + 8 H_2O (water) + 5 O_2 (oxygen). Prior to each fumigation cycle, air was drawn through the impinger train and analyzed as a negative control. In the event that the impinger method and the sensor measurements differed by 10% or greater, standard solutions of HP were prepared and titrated to ensure that the titration method was accurate. The method was adapted from the liquid analysis found at http://www.h2o2.com/intro/highrange.html.

5.2.2 Test Matrix for BIOQUELL Clarus C HP Fumigation

The testing performed with BIOQUELL Clarus C is shown in Table 5-12. The experimental design tested decontamination efficacy by determining whether there was a difference between the log reductions of the viable biological agents after fumigation compared to controls for various materials. These tests also assessed whether there was any difference in efficacy at varying fumigation cycles and fumigation contact times. Critical parameters included fumigation cycle, fumigation contact time, and the viability of the biological agents. An adaptive management approach was used to incorporate new knowledge into the testing as decontamination efficacy results became available.

Table 5-12. Test Matrix for BIOQUELL Clarus C HP Fumigation

Trial	Biological Agent	Material	Fumigation Cycle	Contact Times (min)
1	*B. anthracis* spores*	Carpet	Fumigate 10 min at 8 g/min; dwell at 0.8 g/min	15, 30, 60, 90, 120, 180
2	*B. anthracis* spores*	Carpet	Fumigate 5 min at 8 g/min; dwell at 0.8 g/min	15, 30, 60, 90
3	*B. anthracis* spores*	Carpet	Fumigate 10 min at 8 g/min; dwell at 0.8 g/min	60, 120, 180
4	*Y. pestis**	Aluminum Keyboard Carpet Joint tape	Fumigate 10 min at 8 g/min; dwell at 0.8 g/min	180
5	*B. suis**	Aluminum Keyboard Carpet Joint tape	Fumigate 10 min at 8 g/min; dwell at 0.8 g/min	180
6a	Vaccinia virus*	Keyboard Carpet	Fumigate 10 min at 8 g/min; dwell at 0.8 g/min	180
6b	Vaccinia virus*	Aluminum Joint tape Glass	Fumigate 10 min at 8 g/min; dwell at 0.8 g/min	180
7a	*B. anthracis* spores*	Laminate Ductwork Carpet	Fumigate 10 min at 8 g/min; dwell at 0.8 g/min	180
7b	*B. anthracis* spores*	Concrete Wood Glass Ceiling tile	Fumigate 10 min at 8 g/min; dwell at 0.8 g/min	180

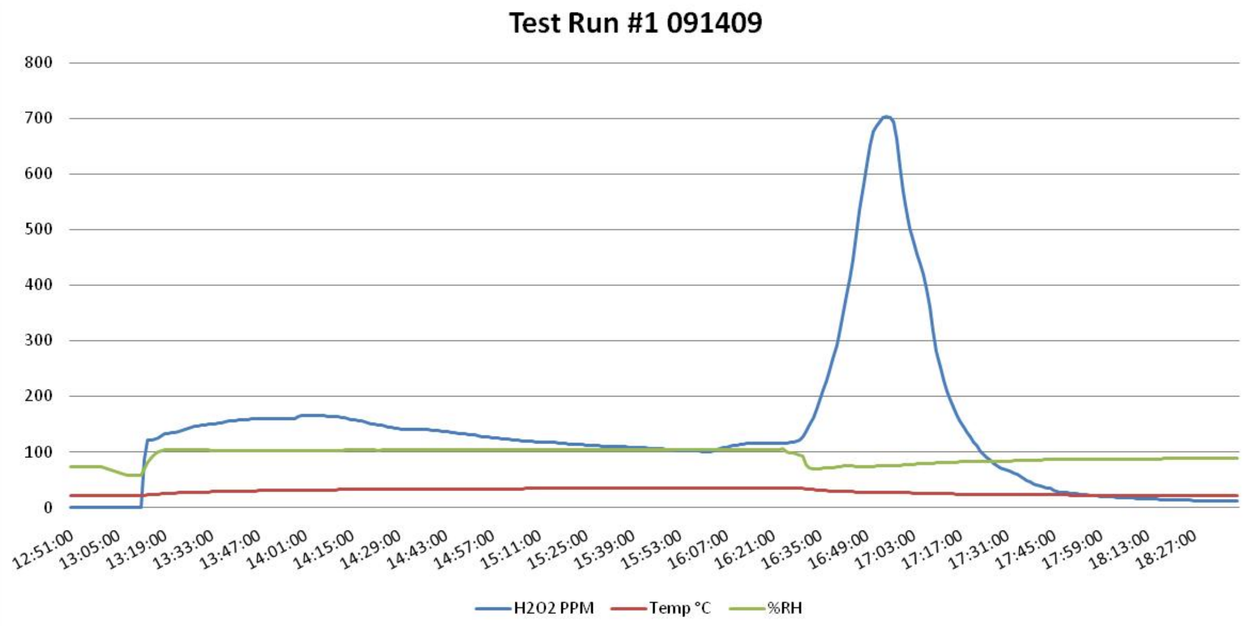
* Biological indicators (*G. stearothermophilus* on steel in Tyvek® envelopes) were also tested.

Five replicate test coupons (plus one blank) per time point and five replicate positive control coupons were included at each set of conditions. Negative controls (blanks) were coupons to which corresponding diluents but no biological agent were applied.

5.2.3 BIOQUELL Clarus C HP Fumigation Results

Figure 5-12 shows a graph of typical temperature, RH, and HP concentration during a fumigation cycle (fumigate 10 min at 8 g/min; dwell at 0.8 g/min). The HP concentration peaked initially and then gradually drifted lower during the dwell phase. The large spike in HP concentration occurred when aeration began and without the introduction of any additional HP. Although the spike was consistently observed, understanding the cause of the spike was beyond the scope of the project and the cause was not determined.

Figure 5-12. Typical Temperature (˚C), RH (%), and HP Concentration (ppmv) Dynamics During the Fumigation Cycle (Fumigate 10 min at 8 g/min; Dwell at 0.8 g/min).



G. stearothermophilus

The results for BIOQUELL Clarus C HP fumigation of biological indicators are shown in Table 5-13. The biological indicators were *G. stearothermophilus* nominally 1 x 10^6 spores on stainless steel in Tyvek® packaging (Apex Laboratories, Apex, NC, USA). In this qualitative test, no growth was observed at any contact time, including the shortest tested (15-min contact).

Table 5-13. BIOQUELL Clarus C HP Fumigation Results for *G. stearothermophilus*

Trial	Fumigation Cycle	Contact Times (min) Growth Positive/Total Biological Indicators					
1,3	Fumigate 10 min at 8 g/min; dwell at 0.8 g/min	15 1/10	30 0/5	60 0/15	90 0/5	120 0/10	180 0/10
2	Fumigate 5 min at 8 g/min; dwell at 0.8 g/min	15 0/10	30 0/5	60 0/5	90 0/5		
4	Fumigate 10 min at 8 g/min; dwell at 0.8 g/min						180 0/10
5	Fumigate 10 min at 8 g/min; dwell at 0.8 g/min						180 0/10
6	Fumigate 10 min at 8 g/min; dwell at 0.8 g/min						180 0/30
7	Fumigate 10 min at 8 g/min; dwell at 0.8 g/min						180 0/10

No growth was observed on the biological indicators following exposure to the fumigant, with the exception of growth observed during trial #1 on one biological indicator following 15 min of exposure to the fumigant. The high efficacy, suggested by the completed kill of the biological indicators, did not correlate with complete kill of *B. anthracis* spores on other materials.

B. anthracis

BIOQUELL Clarus C HP fumigation results for *B. anthracis* spores are presented in Table 5-14.

The efficacy of the BIOQUELL Clarus C HP fumigation varied, depending on the type of test coupon. At 180 min and fumigation cycle of 10 min at 8 g/min; dwell at 0.8 g/min, no viable spores were recovered from laminate, ductwork, glass or ceiling tile. While some efficacy against spores was observed for carpet, concrete, and wood, viable spores were recovered from these materials after fumigation with a 180-min contact time and fumigation cycle of 10 min at 8 g/min; dwell at 0.8 g/min. In one of three replicate trials for carpet for 180 min and fumigation cycle of 10 min at 8 g/min; dwell at 0.8 g/min, no viable spores were recovered.

Table 5-14. BIOQUELL Clarus C HP Fumigation Results for *B. anthracis*

Trial	Contact Time	Material	Spike Amount (CFU/coupon)	Mean Recovered *B. anthracis* (CFU/coupon)*		Mean Log Reduction*
				Positive Control†	Test Coupon‡	
Fumigate 10 min at 8 g/min; dwell at 0.8 g/min						
1	15 min	Carpet	8.57×10^6	$4.50 \pm 0.74 \times 10^6$	$8.03 \pm 5.97 \times 10^5$	0.82 ± 0.26
	30 min	Carpet	8.57×10^6	$4.50 \pm 0.74 \times 10^6$	$2.40 \pm 2.28 \times 10^5$	1.49 ± 0.52
	60 min	Carpet	8.57×10^6	$4.50 \pm 0.74 \times 10^6$	0.00 ± 0.00	6.65 ± 0.00
	90 min	Carpet	8.57×10^6	$4.50 \pm 0.74 \times 10^6$	$5.68 \pm 7.79 \times 10^4$	4.59 ± 2.82
	120 min	Carpet	8.57×10^6	$4.50 \pm 0.74 \times 10^6$	$2.39 \pm 3.33 \times 10^4$	4.74 ± 2.61
	180 min	Carpet	8.57×10^6	$4.50 \pm 0.74 \times 10^6$	0.00 ± 0.00	6.65 ± 0.00
Fumigate 5 min at 4 g/min; dwell at 0.8 g/min						
2	15 min	Carpet	8.17×10^6	$3.27 \pm 1.07 \times 10^6$	$1.85 \pm 1.17 \times 10^6$	0.33 ± 0.32
	30 min	Carpet	8.17×10^6	$3.27 \pm 1.07 \times 10^6$	$1.12 \pm 0.23 \times 10^6$	0.47 ± 0.09
	60 min	Carpet	8.17×10^6	$3.27 \pm 1.07 \times 10^6$	$2.90 \pm 2.60 \times 10^5$	1.62 ± 1.28
	90 min	Carpet	8.17×10^6	$3.27 \pm 1.07 \times 10^6$	$3.84 \pm 1.90 \times 10^5$	0.99 ± 0.27
Fumigate 10 min at 8 g/min; dwell at 0.8 g/min						
3	60 min	Carpet	8.53×10^6	$2.95 \pm 0.26 \times 10^6$	$1.32 \pm 1.81 \times 10^4$	4.14 ± 2.26
	120 min	Carpet	8.53×10^6	$2.95 \pm 0.26 \times 10^6$	$1.66 \pm 1.67 \times 10^5$	3.22 ± 2.97
	180 min	Carpet	8.53×10^6	$2.95 \pm 0.26 \times 10^6$	$2.49 \pm 2.44 \times 10^4$	3.71 ± 2.52
Fumigate 10 min at 8 g/min; dwell at 0.8 g/min						
7a	180 min	Laminate	1.08×10^7	$7.18 \pm 3.60 \times 10^6$	0.00 ± 0.00	6.86 ± 0.00
		Ductwork	1.08×10^7	$2.86 \pm 1.71 \times 10^6$	0.00 ± 0.00	6.46 ± 0.00
		Carpet	1.08×10^7	$5.42 \pm 0.75 \times 10^6$	$8.63 \pm 16.6 \times 10^4$	4.68 ± 2.84
7b	180 min	Concrete	1.08×10^7	$8.51 \pm 2.94 \times 10^6$	$4.99 \pm 10.6 \times 10^3$	5.46 ± 2.07
		Wood	1.08×10^7	$5.25 \pm 1.46 \times 10^5$§	$7.95 \pm 9.18 \times 10^3$	2.16 ± 0.64
		Glass	1.08×10^7	$6.17 \pm 0.72 \times 10^6$	0.00 ± 0.00	6.79 ± 0.00
		Ceiling tile	1.08×10^7	$6.66 \pm 1.63 \times 10^5$§	0.00 ± 0.00	5.82 ± 0.00

* Data are expressed as mean ± standard deviation of five replicates.
† Positive control coupons were spiked but not exposed to the fumigant.
‡ Test coupons were spiked and exposed to the fumigant for the contact time.
§ Below target recovery of ≥10% of spike amount.

B. suis

BIOQUELL Clarus C HP fumigation results for *B. suis* are presented in Table 5-15. No *B. suis* was recovered from aluminum, keyboard, carpet or joint tape following fumigation at a contact time of 180 min and a fumigant cycle that was 10 min at 8 g/min; dwell at 0.8 g/min.

Table 5-15. BIOQUELL Clarus C HP Fumigation Results for *B. suis*

Trial	**Contact Time**	**Material**	**Spike Amount (CFU/ coupon)**	**Mean Recovered *B. suis* (CFU/coupon)***		**Mean Log Reduction***
				Positive Control†	**Test Coupon‡**	
Fumigate 10 min at 8 g/min; dwell at 0.8 g/min						
5	180 min	Aluminum	3.50×10^7	$1.50 \pm 0.64 \times 10^7$	0.00 ± 0.00	7.18 ± 0.00
		Keyboard	3.50×10^7	$2.24 \pm 0.93 \times 10^7$	0.00 ± 0.00	7.35 ± 0.00
		Carpet	3.50×10^7	$7.06 \pm 1.30 \times 10^6$	0.00 ± 0.00	6.85 ± 0.00
		Joint tape	3.50×10^7	$2.17 \pm 0.25 \times 10^6$	0.00 ± 0.00	6.34 ± 0.00

* Data are expressed as mean ± standard deviation of five replicates.
* Data are expressed as mean ± standard deviation of five replicates.
† Positive control coupons were spiked but not exposed to the fumigant.
‡ Test coupons were spiked and exposed to the fumigant for the contact time.

Vaccinia Virus

BIOQUELL Clarus C HP fumigation results for vaccinia virus are presented in Table 5-16. No vaccinia virus was recovered from keyboard, carpet aluminum, joint tape, or glass following fumigation at a contact time of 180 min and a fumigant cycle that was 10 min at 8 g/min; dwell at 0.8 g/min.

Table 5-16. BIOQUELL Clarus C HP Fumigation Results for Vaccinia Virus

Trial	**Contact Time**	**Material**	**Spike Amount (PFUs/ coupon)**	**Mean Recovered *Y. pestis* (PFUs/coupon)***		**Mean Log Reduction***
				Positive Control†	**Test Coupon‡**	
Fumigate 10 min at 8 g/min; dwell at 0.8 g/min						
6a	180 min	Keyboard	3.52×10^7	$7.74 \pm 3.90 \times 10^5$	0.00 ± 0.00	5.89 ± 0.00
		Carpet	3.52×10^7	$3.93 \pm 1.88 \times 10^4$	0.00 ± 0.00	4.59 ± 0.00
6b		Aluminum	9.64×10^7	$1.59 \pm 0.59 \times 10^7$	0.00 ± 0.00	7.20 ± 0.00
		Joint tape	4.30×10^7	$1.09 \pm 0.45 \times 10^5$	0.00 ± 0.00	5.04 ± 0.00
		Glass	9.64×10^7	$1.60 \pm 0.54 \times 10^7$	0.00 ± 0.00	7.20 ± 0.00

* Data are expressed as mean ± standard deviation of five replicates.
† Positive control coupons were spiked but not exposed to the fumigant.
‡ Test coupons were spiked and exposed to the fumigant for the contact time.

Y. pestis

BIOQUELL Clarus C HP fumigation results for *Y. pestis* are presented in Table 5-17. No *Y. pestis* was recovered from keyboard, carpet aluminum, joint tape, or glass following fumigation at a contact time of 180 min and a fumigant cycle that was 10 min at 8 g/min; dwell at 0.8 g/min.

Table 5-17. BIOQUELL Clarus C HP Fumigation Results for *Y. pestis*

Trial	Contact Time	Material	Spike Amount (CFUs/ coupon)	Mean Recovered *Y. pestis* (CFUs/coupon)*		Mean Log Reduction*
				Positive Control†	Test Coupon‡	
Fumigate 10 min at 8 g/min; dwell at 0.8 g/min						
4	180 min	Aluminum	9.07×10^6	$3.02 \pm 0.71 \times 10^4$	0.00 ± 0.00	4.48 ± 0.00
		Keyboard	9.07×10^6	$4.56 \pm 1.53 \times 10^5$	0.00 ± 0.00	5.66 ± 0.00
		Carpet	9.07×10^6	$2.14 \pm 0.93 \times 10^3$	0.00 ± 0.00	3.33 ± 0.00
		Joint tape	9.07×10^6	$4.29 \pm 2.76 \times 10^3$	0.00 ± 0.00	3.63 ± 0.00

* Data are expressed as mean ± standard deviation of five replicates.
† Positive control coupons were spiked but not exposed to the fumigant.
‡ Test coupons were spiked and exposed to the fumigant for the contact time.

Summary Statistics for BIOQUELL Clarus C HP Decontamination

Table 5-18 provides a summary of BIOQUELL Clarus C HP Fumigation decontamination efficacy, calculated as the difference in the mean log of viable bacteria recovered from positive control coupons and the mean log of viable bacteria recovered from coupons after fumigation for a given contact time. The elapsed time from spiking to recovery was the same for the positive control coupons and test coupons to control for history. The 95% CI and p-value are also shown. At the 180-min contact time (fumigate 10 min at 8 g/min; dwell at 0.8 g/min), no viable *Y. pestis*, *B. suis*, or vaccinia were recovered from any type of material tested. At the 180-min contact time (fumigate 10 min at 8 g/min; dwell at 0.8 g/min), *B. anthracis* was recovered only from carpet, concrete, and wood.

Surface Damage

The physical effect of the BIOQUELL Clarus C HP fumigation on the materials was evaluated qualitatively. The appearance of the decontaminated coupons was visually inspected for any obvious changes in the color, reflectivity, and apparent roughness of the material surfaces. These comparisons were performed for each material, before extraction of the decontaminated test coupons. No differences were observed between control and fumigated coupons for any material, except that the coupons were visibly moistened by condensation during fumigation.

Table 5-18. Summary of Efficacy (Calculated as Mean Log Reduction) of BIOQUELL Clarus C HP Fumigation

Trials	Agent	Material	Mean Log Reduction (95% CI) and p-Value* or Mean Log Reduction (# of treated coupons with zero recovery/# of treated coupons) and p-Value†					
			15 min	**30 min**	**60 min**	**90 min**	**120 min**	**180 min**
Trial 1	*B. anthracis* spores	Carpet	**0.82 (0.54, 1.10) p=0.0015**	**1.48 (0.95, 2.02) p=0.0027**	**>6.65 (5/5) p=0.0079**	**>4.59 (3/5) p=0.0079**	**>4.74 (3/5) p=0.0079**	**>6.65 (5/5) p=0.0079**
Trial 2	*B. anthracis* spores	Carpet	0.31 (-0.06, 0.69) p=0.1032	**0.45 (0.26, 0.64) p=0.0014**	**1.60 (0.27, 2.93) p=0.0486**	**0.97 (0.64, 1.29) p=0.0003**		
Trial 3	*B. anthracis* spores	Carpet			**>4.14 (2/5) p=0.0079**		**>3.22 (2/5) p=0.0079**	**>3.71 (2/5) p=0.0079**
Trial 4	*Y. pestis*	Aluminum						**>4.47 (5/5) p=0.0079**
		Carpet						**>3.30 (5/5) p=0.0079**
		Keyboard						**>5.64 (5/5) p=0.0079**
		Painted Joint Tape						**>3.54 (5/5) p=0.0079**
Trial 5	*B. suis*	Aluminum						**>7.15 (5/5) p=0.0079**
		Carpet						**>6.84 (5/5) p=0.0079**
		Keyboard						**>7.31 (5/5) p=0.0079**
		Painted Joint Tape						**>6.33 (5/5) p=0.0079**
Trial 6	Vaccinia	Aluminum						**>7.18 (5/5) p=0.0079**
		Carpet						**>4.55 (5/5) p=0.0079**
		Glass						**>7.18 (5/5) p=0.0079**
		Keyboard						**>5.85 (5/5) p=0.0079**
		Painted Joint Tape						**>5.00 (5/5) p=0.0079**
Trial 7	*B. anthracis* spores	Carpet						**>4.67 (3/5) p=0.0079**
		Ceiling Tile						**>5.81 (5/5) p=0.0079**
		Concrete						**>5.43 (3/5) p=0.0079**
		Glass						**>6.79 (5/5) p=0.0079**
		Laminate						**>6.81 (5/5) p=0.0079**
		Metal						**>6.34 (5/5) p=0.0079**
		Wood						**2.15 (1.48, 2.82) p=0.0014**

* Mean log reduction is the mean of the base-10 logarithm of recovered agent from the control coupons minus the mean of the base-10 logarithm of recovered agent from the treated coupons. A 95 % CI for the difference is shown in parentheses. A p-value is provided for the probability that the control and treatment recoveries are the same. The p-value is from the two sample t-test with Satterthwaite's method to allow for potentially different variances in the two groups. p-Values less than 0.05 denote less than 1 in 20 chance that a difference as large as or larger than observed would occur by chance if the control and treatment means were truly identical. Comparisons with p-values less than 0.05 (statistically significant at the 0.05 level) are bolded.

† One or more of the treatment coupons had no recovered agent. The mean log reduction of the form ">X" is calculated as the mean of the base-10 logarithm of recovered agent from the control coupons minus the mean of the base-10 logarithm of recovered agent from the treated coupons except that "zero recovery" coupons have a substituted recovered value of "1" (base-10 log is 0). Since the log becomes an increasingly negative value below 1 and is undefined at 0, this substitution is necessary and results in a lower bound on the mean log difference, as indicated by the ">". The number of "zero recovery" treatment coupons and the total number of treatment coupons is shown in parentheses. The p-value is from the non-parametric Kolmogorov-Smirnov test. p-Values less than 0.05 denote less than 1 in 20 chance that results as different as or more different than observed would occur by chance if the distribution of the control and treatment recoveries were truly identical. Comparisons with p-values less than 0.05 (statistically significant at the 0.05 level) are bolded.

5.3 HP Fumigation (BIOQUELL Clarus S)

5.3.1 Description of BIOQUELL Clarus S HP Technology

The BIOQUELL Clarus S, shown in Figure 5-13, is a compact and mobile HP vapor technology designed to bio-decontaminate laboratory equipment. BIOQUELL's Clarus S uses low temperature, residue-free hydrogen peroxide vapor technology. The BIOQUELL Clarus S Hydrogen Peroxide Vapor Generation System was operated in an automated cycle controlled by the commercial unit using manufacturer's recommended parameters. The Clarus S unit controlled the cycle phases and duration.

Figure 5-13. BIOQUELL Clarus S HP Vapor Generator.[15]

The HP vapor in the test chamber was monitored using an Analytical Technology HP gas sensor with liquid crystal display, as described in Section 5.2.1. The HP gas sensor was oriented in the test chamber at a position distant to the HP vaporizer but in proximity to test coupons. The display unit and power supply were located outside the test chamber. The concentration of HP vapor was documented approximately every 20 min during the gassing portion of the decontamination cycle while test coupons are in the test chamber. The HP vapor concentration was a non-critical measurement, but the data from the sensor enabled monitoring of HP concentration variability between fumigation cycles.

5.3.2 Test Matrix for BIOQUELL Clarus S HP Fumigation

The testing performed with BIOQUELL Clarus S fumigation is shown in Table 5-19. The experimental design tested decontamination efficacy by determining whether there was a difference between the log reductions in the viable biological agents after fumigation compared to controls for various materials. These tests also assessed whether there was any difference in efficacy at varying RH and fumigation contact times. Critical parameters impacting the viability of biological agents amount of HP injected, fumigation contact time, temperature, RH, and natural attenuation. An adaptive management approach was used to incorporate new knowledge into the testing as decontamination efficacy results became available.

Table 5-19. Test Matrix for BIOQUELL Clarus S HP Fumigation

Trial	Biological Agent	Material	HP Fumigation Parameters	Initial Environmental Condition	Contact Times (min)
1	*G. stearothermophilus*	Biological indicator: Steel in Tyvek®	HP volume: 15 mL Injection time: 55 min	40% - 50% RH; 22 °C ± 3 °C (ambient)	5, 30, 120
2	*F. tularensis*	Aluminum Keyboard Carpet Joint tape	HP volume: 15 mL Injection time: 15 min	40% - 50% RH; 22 °C ± 3 °C (ambient)	30, 60
3	*F. tularensis*	Aluminum Keyboard Carpet Joint tape	HP volume: 15 mL Injection time: 15 min	60% - 70% RH; 22 °C ± 3 °C	15*, 30, 60
4	*B. suis*	Aluminum Keyboard Carpet Joint tape	HP volume: 15 mL Injection time: 15 min	40% - 50% RH; 22 °C ± 3 °C (ambient)	15, 30
5	*B. suis*	Aluminum Keyboard Carpet Joint tape	HP volume: 15 mL Injection time: 15 min	60% - 70% RH; 22 °C ± 3 °C (ambient)	15, 30, 60
6	*Y. pestis*	Aluminum Keyboard Carpet Joint tape	HP volume: 15 mL Injection time: 15 min	40% - 50% RH; 22 °C ± 3 °C (ambient)	15, 30
7	*Y. pestis*	Aluminum Keyboard Carpet Joint tape	HP volume: 15 mL Injection time: 15 min	60% - 70% RH; 22 °C ± 3 °C (ambient)	15, 30, 60
8	*B. anthracis* spores	Keyboard Carpet	HP volume: 15 mL Injection time: 15 min	40% - 50% RH; 22 °C ± 3 °C (ambient)	30, 60
9	*B. anthracis* spores	Carpet	HP volume: 15 mL Injection time: 15 min Dwell 45: min HP volume: 17.5 mL Injection time: 15 min Dwell 45: min HP volume: 17.5 mL Injection time: 15 min Dwell 45: min	40% - 50% RH; 22 °C ± 3 °C (ambient)	60, 126, 192
9a	*B. anthracis* spores	Aluminum Keyboard Carpet Joint tape	HP volume: 50 mL Injection time: 20 min	40% - 50% RH; 2 2ºC ± 3 ºC (ambient)	75

*Carpet and joint tape only.

5.3.3 BIOQUELL Clarus S HP Fumigation Results

Figure 5-14 shows a graph of typical temperature, RH, and HP concentration during a fumigation cycle (15 mL HP injection). The HP concentration peaked initially, and then gradually drifted lower during the dwell phase (starting at 15 minutes in Figure 5-14, after the injection phase).

Figure 5-14. Typical Temperature (°C), RH (%), and HP Concentration (ppmv) Dynamics During the Fumigation Cycle (15 mL HP Injection).

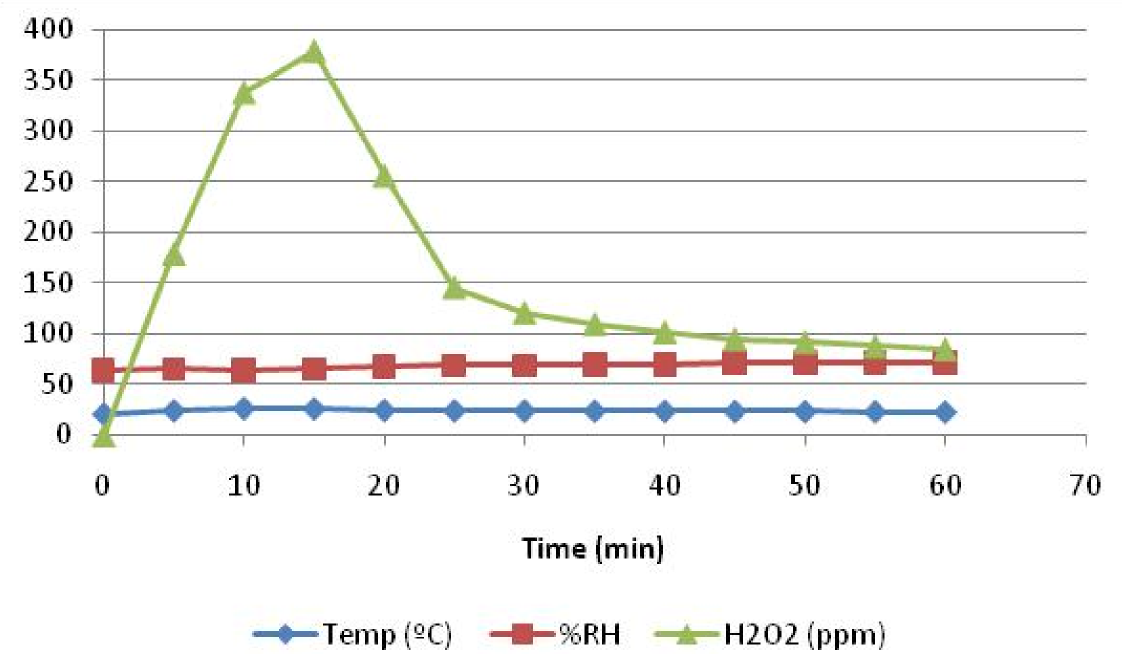
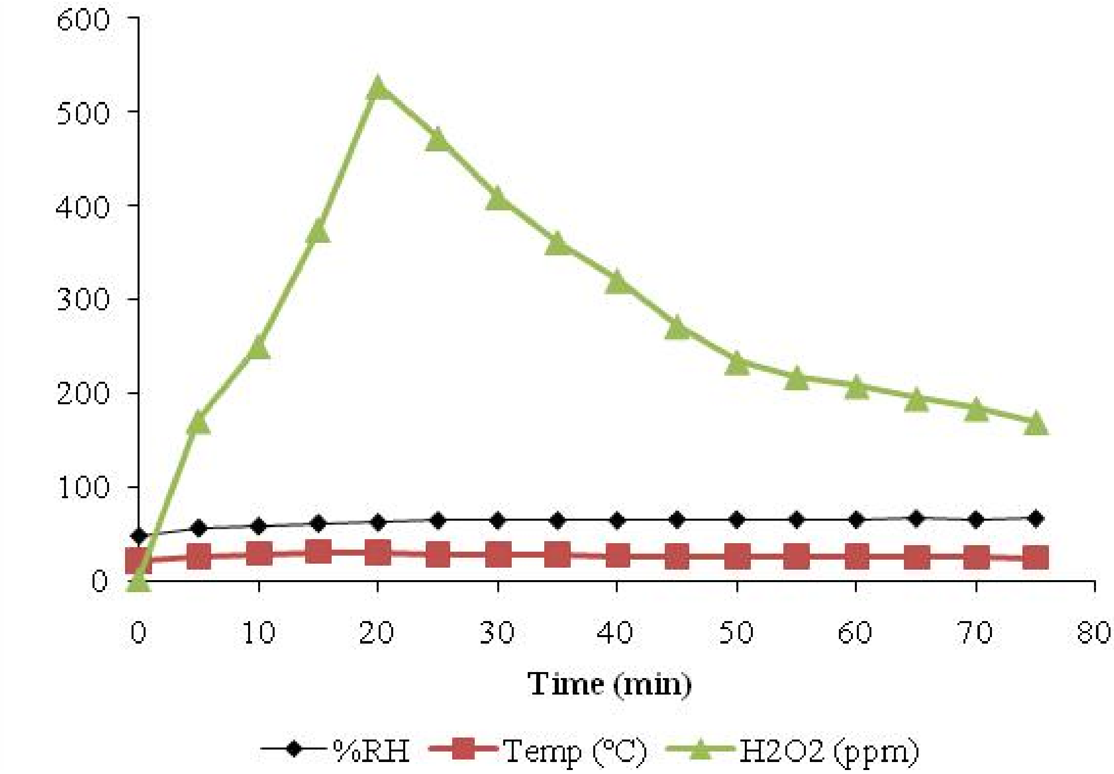


Figure 5-15 shows a graph of temperature, RH, and HP concentration during a high concentration fumigation cycle (50 mL HP injection). The HP concentration peaked initially, and then gradually drifted lower during the dwell phase (starting at 20 minutes in Figure 5-14, after the injection phase).

Figure 5-15. Typical Temperature (°C), RH (%), and HP Concentration (ppmv) Dynamics During the Fumigation cycle (50 mL HP Injection).



G. stearothermophilus

The results for BIOQUELL Clarus S HP fumigation of biological indicators are shown in Table 5-20. The biological indicators were *G. stearothermophilus* nominally 1 x 10^6 spores on stainless steel in Tyvek® packaging. In this qualitative test, no growth was observed at any contact time, including the shortest time tested (5-min contact time).

Table 5-20. BIOQUELL Clarus S HP Fumigation Results for *G. stearothermophilus*

Trial	Fumigation Cycle	Contact Times (min) Growth Positive/Total Biological Indicators		
1	HP volume: 15 mL Injection time: 55 min	5 0/5	30 0/5	120 0/10

No growth was observed on the biological indicators following exposure to the fumigant. The high efficacy, suggested by the completed kill of the biological indicators, did not correlate with complete kill of *B. anthracis* spores on other materials.

B. anthracis

BIOQUELL Clarus S HP fumigation results for *B. anthracis* spores are presented in Table 5-21. With an HP volume of 15 mL injected over 15 min and a contact time of 30 min, no viable *B. anthracis* spores were recovered from keyboard (initial RH 40% - 50%). No viable *B. anthracis* spores were recovered from aluminum or joint tape with an HP volume of 50 mL injected over 20-min and a 75-min contact time (initial RH 40% - 50%). Note that 75 min was the shortest contact time tested with aluminum and joint tape. In contrast, lower efficacy was observed against *B. anthracis* spores on carpet; viable spores were recovered from the carpet after a 192-min contact time (with a cycle including multiple HP injections totaling 50 mL, initial RH: 40% - 50%; see Table 5-19). Viable *B. anthracis* spores were also recovered after a 75-min contact time with a fumigant cycle of 50 mL HP injected over 20 min (initial RH: 40% - 50%).

Table 5-21. BIOQUELL Clarus S HP Fumigation Results for *B. anthracis*

Trial	Contact Time	Material	Spike Amount (CFU/coupon)	Mean Recovered *B. anthracis* (CFU/coupon)*		Mean Log Reduction*
				Positive Control†	Test Coupon‡	
Initial RH 45%; Fumigate 15 mL Injected Over 15 min						
8	30 min	Keyboard	3.03 x 10^6 §	1.40 ± 0.44 x 10^6	0.00 ± 0.00	6.14 ± 0.00
		Carpet	3.03 x 10^6 §	5.88 ± 0.50 x 10^6	9.83 ± 3.94 x 10^5	0.80 ± 0.16
8	60 min	Keyboard	3.03 x 10^6 §	1.24 ± 0.33 x 10^6	0.00 ± 0.00	6.09 ± 0.00
		Carpet	3.03 x 10^6 §	3.94 ± 2.24 x 10^6	1.16 ± 0.44 x 10^6	0.56 ± 0.19
Initial RH 45%; Three Fumigate Cycles Totaling 50 mL Injected Over 3 x 15 min Dwell between injections ~45 min						
9	60 min	Carpet	2.33 x 10^6 §	4.35 ± 0.42 x 10^6	9.74 ± 3.81 x 10^5	0.68 ± 0.17
	126 min	Carpet	2.33 x 10^6 §	4.35 ± 0.42 x 10^6	5.00 ± 7.62 x 10^4	3.82 ± 2.61
	192 min	Carpet	2.33 x 10^6 §	4.35 ± 0.42 x 10^6	1.61 ± 3.34 x 10^4	4.92± 2.39
Initial RH 45%; Fumigate 50 mL Injected Over 20 min						
9a	75 min	Aluminum	8.40 x 10^6	1.26 ± 0.84 x 10^5	0.00 ± 0.00	5.10 ± 0.00
		Keyboard	8.40 x 10^6	1.81 ± 2.32 x 10^5	0.00 ± 0.00	5.26 ± 0.00
		Carpet	8.40 x 10^6	4.70 ± 0.55 x 10^6	9.81 ± 7.64 x 10^5	0.81 ± 0.41
		Joint tape	8.40 x 10^6	1.83 ± 0.76 x 10^6	0.00 ± 0.00	6.26 ± 0.00

* Data are expressed as mean ± standard deviation of five replicates.
† Positive control coupons were spiked but not exposed to the fumigant.
‡ Test coupons were spiked and exposed to the fumigant for the contact time.
§ Application was lower than the target 7.5 x 106 - 1.25 x 107 CFUs/coupon.

B. suis

BIOQUELL Clarus S HP fumigation results for *B. suis* are presented in Table 5-22. Efficacy against *B. suis* was highly variable, depending on the type of material. Viable bacteria were recovered from the aluminum, keyboard, and carpet after a 60-min contact time and a fumigation cycle of 15 mL injected over 15-min (65% initial RH). However, under these conditions, no viable bacteria were recovered from joint tape. Part of the loss of viable bacteria from joint tape was attributable to possible bactericidal activity of the paint or joint tape, as shown by the decrease in viable bacteria recovered from the positive control coupons after the 60-min drying time and the additional 60 min time before extraction, corresponding to the CT. The impact of HP fumigation was still discernible.

As described in the Introduction, the calculated log reductions reflect the incremental impact of the fumigation technology. Compared to the amount of bacteria spiked onto the coupon (6.57×10^7 CFUs), recovery of no viable bacteria from the joint tape after the 60-min treatment represents >7 log reduction in viable bacteria attributable to the fumigation and the loss of viability from joint tape arising from other (unknown) causes.

Table 5-22. BIOQUELL Clarus S HP Fumigation Results for *B. suis*

Trial	Contact Time	Material	Spike Amount (CFU/coupon)	Mean Recovered *B. suis* (CFU/coupon)*		Mean Log Reduction*
				Positive Control†	Test Coupon‡	
Initial RH 45%; Fumigate 15 mL Injected Over 15 min						
4	15 min	Aluminum	9.93×10^7	$3.79 \pm 0.88 \times 10^7$	$5.54 \pm 4.32 \times 10^4$	2.95 ± 0.35
		Keyboard	9.93×10^7	$2.76 \pm 0.55 \times 10^7$	$1.96 \pm 3.93 \times 10^5$	3.20 ± 1.25
		Carpet	1.77×10^{8}§	$2.35 \pm 0.45 \times 10^8$	$1.41 \pm 0.09 \times 10^8$	0.22 ± 0.03
		Joint tape	1.77×10^{8}§	$2.48 \pm 1.97 \times 10^6$	$1.10 \pm 1.94 \times 10^5$	2.55 ± 1.45
4	30 min	Aluminum	9.93×10^7	$3.65 \pm 1.21 \times 10^7$	$1.27 \pm 2.31 \times 10^2$	6.62 ± 1.32
		Keyboard	9.93×10^7	$2.13 \pm 0.40 \times 10^7$	0.00 ± 0.00	7.33 ± 0.00
		Carpet	1.77×10^{8}§	$2.33 \pm 0.24 \times 10^8$	$1.11 \pm 0.26 \times 10^8$	0.33 ± 0.10
		Joint tape	1.77×10^{8}§	$1.66 \pm 1.47 \times 10^6$	$3.63 \pm 3.27 \times 10^5$	0.79 ± 0.38
Initial RH 65%; Fumigate 15 mL Injected Over 15 min						
5	15 min	Aluminum	3.10×10^7	$2.65 \pm 0.31 \times 10^7$	$4.70 \pm 1.56 \times 10^6$	0.77 ± 0.16
		Keyboard	3.10×10^7	$3.10 \pm 0.59 \times 10^7$	$2.49 \pm 0.77 \times 10^6$	1.11 ± 0.13
		Carpet	6.57×10^7	$1.35 \pm 1.32 \times 10^7$	$1.25 \pm 0.85 \times 10^6$	1.20 ± 0.51
		Joint tape	6.57×10^7	$1.98 \pm 2.18 \times 10^5$	$7.40 \pm 14.3 \times 10^0$	4.99 ± 0.68
5	30 min	Aluminum	3.10×10^7	$3.20 \pm 0.94 \times 10^7$	$2.37 \pm 0.92 \times 10^6$	1.17 ± 0.21
		Keyboard	3.10×10^7	$3.20 \pm 0.60 \times 10^7$	$1.22 \pm 1.26 \times 10^6$	1.68 ± 0.58
		Carpet	6.57×10^7	$1.01 \pm 0.66 \times 10^7$	$1.09 \pm 1.19 \times 10^3$	4.38 ± 0.82
		Joint tape	6.57×10^7	$8.25 \pm 8.05 \times 10^3$	$2.40 \pm 5.20 \times 10^4$	1.24 ± 1.85
5	60 min	Aluminum	3.10×10^7	$7.94 \pm 3.60 \times 10^7$	$1.50 \pm 1.24 \times 10^6$	1.90 ± 0.50
		Keyboard	3.10×10^7	$2.11 \pm 0.38 \times 10^7$	$2.71 \pm 3.59 \times 10^5$	2.27 ± 0.74
		Carpet	6.57×10^7	$1.32 \pm 1.78 \times 10^6$	$2.81 \pm 6.26 \times 10^2$	5.49 ± 1.41
		Joint tape	6.57×10^7	$2.79 \pm 2.21 \times 10^3$	0.00 ± 0.00	3.45 ± 0.00

* Data are expressed as mean ± standard deviation of five replicates.
† Positive control coupons were spiked but not exposed to the fumigant.
‡ Test coupons were spiked and exposed to the fumigant for the contact time.
§ Application exceeded the target 1.0×10^6 - 1.0×10^8 CFU/coupon.

F. tularensis

BIOQUELL Clarus S HP fumigation results for *F. tularensis* are presented in Table 5-23. The Clarus S HP fumigation was efficacious for all materials at the shortest time used and independent of the starting RH values investigated. No *F. tularensis* was recovered from carpet or joint tape following fumigation at a contact time of 15 min and a fumigation cycle of 15 mL injected over 15 min (65% initial RH). No *F. tularensis* was recovered from aluminum or keyboard following fumigation at a contact time of 30 min (shortest contact time tested) and a fumigation cycle of 15 mL injected over 15 min (45% or 65% initial RH). As described in the Introduction, the calculated log reductions reflect the incremental impact of the fumigation technology. Compared to the amount of bacteria spiked onto the coupon (about 107 CFUs/coupon), after a 15-min treatment of *F. tularensis* on carpet or joint tape or a 30 min treatment of *F. tularensis* on aluminum and keyboard a >7 log reduction in viable bacteria was attributable to the fumigation and the loss of viability arising from other (unknown) time-dependent causes.

Table 5-23. BIOQUELL Clarus S HP Fumigation Results for *F. tularensis*

Trial	Contact Time	Material	Spike Amount (CFU/ coupon)	Mean Recovered *F. tularensis* (CFU/coupon)*		Mean Log Reduction*
				Positive Control†	Test Coupon‡	
Initial RH 45%; Fumigate 15 mL Injected Over 15 min						
2	30 min	Aluminum	1.33×10^7	$1.61 \pm 0.51 \times 10^5$	0.00 ± 0.00	5.21 ± 0.00
		Keyboard	1.33×10^7	$3.80 \pm 1.55 \times 10^5$	0.00 ± 0.00	5.58 ± 0.00
		Carpet	1.58×10^7	$5.09 \pm 1.52 \times 10^6$	0.00 ± 0.00	6.71 ± 0.00
		Joint tape	1.58×10^7	$8.67 \pm 12.1 \times 10^2$	0.00 ± 0.00	2.94 ± 0.00
2	60 min	Aluminum	1.33×10^7	$6.27 \pm 4.16 \times 10^4$	0.00 ± 0.00	4.80 ± 0.00
		Keyboard	1.33×10^7	$9.63 \pm 1.29 \times 10^4$	0.00 ± 0.00	4.98 ± 0.00
		Carpet	1.58×10^7	$8.96 \pm 6.44 \times 10^5$	0.00 ± 0.00	5.95 ± 0.00
		Joint tape	1.58×10^7	$2.83 \pm 1.23 \times 10^2$	0.00 ± 0.00	2.45 ± 0.00
Initial RH 65%; Fumigate 15 mL Injected Over 15 min						
3	15 min	Carpet	1.77×10^7	$3.30 \pm 2.26 \times 10^6$	0.00 ± 0.00	6.52 ± 0.00
		Joint tape	1.77×10^7	$1.38 \pm 1.75 \times 10^1$	0.00 ± 0.00	1.14 ± 0.00
3	30 min	Carpet	1.77×10^7	$3.00 \pm 0.69 \times 10^6$	0.00 ± 0.00	6.48 ± 0.00
		Joint tape	1.77×10^7	$4.01 \pm 4.82 \times 10^3$	0.00 ± 0.00	3.60 ± 0.00
3	60 min	Carpet	1.77×10^7	$1.01 \pm 1.92 \times 10^4$	0.00 ± 0.00	4.00 ± 0.00
		Joint tape	1.77×10^7	0.00 ± 0.00	0.00 ± 0.00	Not calculable
Initial RH 65%; Fumigate 15 mL Injected Over 15 min						
3	30 min	Aluminum	1.65×10^7	$6.63 \pm 2.76 \times 10^4$	0.00 ± 0.00	4.82 ± 0.00
		Keyboard	1.65×10^7	$1.30 \pm 1.21 \times 10^6$	0.00 ± 0.00	6.11 ± 0.00
3	60 min	Aluminum	1.65×10^7	$1.91 \pm 1.70 \times 10^4$	0.00 ± 0.00	4.28 ± 0.00
		Keyboard	1.65×10^7	$3.85 \pm 3.50 \times 10^5$	0.00 ± 0.00	5.59 ± 0.00
3	90 min	Aluminum	1.65×10^7	$6.98 \pm 2.34 \times 10^3$	0.00 ± 0.00	3.84 ± 0.00
		Keyboard	1.65×10^7	$1.04 \pm 0.30 \times 10^5$	0.00 ± 0.00	5.02 ± 0.00

* Data are expressed as mean ± standard deviation of five replicates.
† Positive control coupons were spiked but not exposed to the fumigant.
‡ Test coupons were spiked and exposed to the fumigant for the contact time.

Y. pestis

BIOQUELL Clarus S HP fumigation results for *Y. pestis* are presented in Table 5-24. Efficacy against *Y. pestis* was highly variable, depending on the type of material. Viable bacteria were recovered from the aluminum, keyboard, and carpet after a 60-min contact time and a fumigation cycle of 15 mL injected over 15 min (65% initial RH). However, under these conditions, no viable bacteria were recovered from joint tape. Part of the loss of viable bacteria from joint tape was attributable to possible bactericidal activity of the paint or joint tape, as shown by the decrease in viable bacteria recovered from the positive control coupons. As described in the Introduction, the calculated log reductions reflect the incremental impact of the fumigation technology. Compared to the amount of bacteria spiked onto the coupon (about 10^7 CFUs/coupon), after a 60-min treatment of *Y. pestis* on the various types of coupons, efficacy ranging from <1 log reduction to >7 log reduction in viable bacteria was attributable to the combined effects of fumigation and the loss of viability arising from other (unknown) time-dependent causes.

Table 5-24. BIOQUELL Clarus S HP Fumigation Results for *Y. pestis*

Trial	Contact Time	Material	Spike Amount (CFU/coupon)	Mean Recovered *Y. pestis* (CFU/coupon)*		Mean Log Reduction*
				Positive Control†	Test Coupon‡	
Initial RH 45%; Fumigate 15 mL Injected Over 15 min						
6	15 min	Aluminum	4.93×10^7	$1.53 \pm 0.87 \times 10^5$	$1.40 \pm 2.91 \times 10^1$	4.82 ± 0.81
		Keyboard	4.93×10^7	$4.02 \pm 2.53 \times 10^4$	$2.67 \pm 3.60 \times 10^2$	2.72 ± 1.11
		Carpet	6.03×10^7	$6.47 \pm 5.86 \times 10^6$	$1.66 \pm 2.58 \times 10^7$	0.11 ± 0.84
		Joint tape	6.03×10^7	$1.77 \pm 1.86 \times 10^4$	$3.40 \pm 4.67 \times 10^1$	3.48 ± 1.05
6	30 min	Aluminum	4.93×10^7	$3.41 \pm 2.11 \times 10^4$	0.00 ± 0.00	4.53 ± 0.00
		Keyboard	4.93×10^7	$2.92 \pm 1.19 \times 10^5$	$7.36 \pm 12.8 \times 10^1$	4.36 ± 1.08
		Carpet	6.03×10^7	$1.14 \pm 0.28 \times 10^8$	$2.03 \pm 3.53 \times 10^6$	3.23 ± 1.88
		Joint tape	6.03×10^7	$8.41 \pm 5.20 \times 10^3$	$3.58 \pm 5.08 \times 10^2$	2.46 ± 1.47
Initial RH 65%; Fumigate 15 mL Injected Over 15 min						
7	15 min	Aluminum	5.27×10^7	$1.02 \pm 0.46 \times 10^5$	$7.46 \pm 3.92 \times 10^2$	2.21 ± 0.31
		Keyboard	5.27×10^7	$9.97 \pm 6.79 \times 10^4$	$1.31 \pm 2.02 \times 10^4$	1.18 ± 0.50
		Carpet	5.03×10^7	$6.43 \pm 3.28 \times 10^7$	$3.39 \pm 1.35 \times 10^7$	0.30 ± 0.17
		Joint tape	5.03×10^7	$5.80 \pm 5.91 \times 10^6$	$6.01 \pm 6.12 \times 10^5$	1.26 ± 0.61
7	30 min	Aluminum	5.27×10^7	$1.66 \pm 0.89 \times 10^4$	$4.07 \pm 5.01 \times 10^2$	2.24 ± 1.21
		Keyboard	5.27×10^7	$1.52 \pm 0.28 \times 10^5$	$6.09 \pm 4.95 \times 10^3$	1.52 ± 0.37
		Carpet	5.03×10^7	$3.60 \pm 2.81 \times 10^7$	$3.29 \pm 1.40 \times 10^7$	0.08 ± 0.22
		Joint tape	5.03×10^7	$1.79 \pm 2.91 \times 10^3$	$2.15 \pm 4.78 \times 10^2$	2.65 ± 1.35
7	60 min	Aluminum	5.27×10^7	$1.13 \pm 0.28 \times 10^5$	$1.26 \pm 1.59 \times 10^1$	4.46 ± 0.81
		Keyboard	5.27×10^7	$2.41 \pm 0.44 \times 10^4$	$2.92 \pm 1.92 \times 10^2$	2.02 ± 0.38
		Carpet	5.03×10^7	$2.49 \pm 0.67 \times 10^7$	$1.83 \pm 0.58 \times 10^7$	0.15 ± 0.15
		Joint tape	5.03×10^7	$2.18 \pm 2.82 \times 10^2$	0.00 ± 0.00	2.34 ± 0.00

* Data are expressed as mean ± standard deviation of five replicates.
† Positive control coupons were spiked but not exposed to the fumigant.
‡ Test coupons were spiked and exposed to the fumigant for the contact time.

Summary Statistics for BIOQUELL Clarus S HP Decontamination

Table 5-25 provides a summary of BIOQUELL Clarus S HP fumigation efficacy results. Because of the loss of viable *Y. pestis* from positive control coupons due to unknown, time-dependent causes, the log reductions attributable only to the fumigation effect may appear low or may not be determined. The combined effects attributable to the 60 min fumigation treatment and the loss of viability over time from other (unknown) causes result in >7 log reduction in viable *F. tularensis* bacteria applied to the test coupons. For *B. suis* and *Y. pestis*, the efficacies varied depending on the type of coupon material and the CT. The BIOQUELL Clarus S HP fumigation treatment showed high efficacy (no recovered spores) against *B. anthracis*, except the treatment showed low efficacy against spores on aluminum. A reaction between the HP and aluminum may explain the results.

Surface Damage

The physical effect of the BIOQUELL Clarus S HP fumigation on the materials was evaluated qualitatively. The appearance of the decontaminated coupons was visually inspected for any obvious changes in the color, reflectivity, and apparent roughness of the material surfaces. These comparisons were performed for each material, before extraction of the decontaminated test coupons. No differences were observed between control and fumigated coupons for any material, except that the coupons were visibly moistened by condensation during fumigation.

Table 5-25. Summary of Efficacy (Calculated as Mean Log Reduction) of BIOQUELL Clarus S HP Fumigation Results

Trials	Agent	Material	Mean Log Reduction (95% CI) and p-Value* or Mean Log Reduction (# of treated coupons with zero recovery/# of treated coupons) and p-Value† or N/A (# of control coupons with zero recovery/# of control coupons # of treated coupons with zero recovery/# of treated coupons) and p-Value‡				
			15 min	**30 min**	**60 min**	**75 min**	**90 min**
Trial 2	*F. tularensis*	Aluminum		**>5.19 (5/5) p=0.0079**	**>4.46 (5/5) p=0.0079**		
		Carpet		**>6.69 (5/5) p=0.0079**	**>5.83 (5/5) p=0.0079**		
		Keyboard		**>5.55 (5/5) p=0.0079**	**>4.98 (5/5) p=0.0079**		
		Painted Joint Tape		**>2.63 (5/5) p=0.0079**	**>2.42 (4/4) p=0.0286**		
Trial 3	*F. tularensis*	Aluminum		**>4.78 (5/5) p=0.0079**	**>4.06 (5/5) p=0.0079**		**>3.82 (5/5) p=0.0079**
		Carpet	**>6.45 (5/5) p=0.0079**	**>6.47 (5/5) p=0.0079**	**>3.19 (5/5) p=0.0079**		
		Keyboard		**>5.95 (5/5) p=0.0079**	**>5.44 (5/5) p=0.0079**		**>5.00 (5/5) p=0.0079**
		Painted Joint Tape	N/A (3/5 5/5) p=0.4444	N/A (1/5 5/5) p=0.0476	N/A (5/5 5/5) p=1.0000		
Trial 4	*B. suis*	Aluminum	**2.94 (2.56, 3.32) p<0.0001**	**>6.60 (3/5) p=0.0079**			
		Carpet	**0.22 (0.12, 0.31) p=0.0032**	**0.33 (0.21, 0.45) p=0.0010**			
		Keyboard	**3.19 (1.90, 4.49) p=0.0046**	**>7.32 (5/5) p=0.0079**			
		Painted Joint Tape	**2.47 (0.94, 3.99) p=0.0180**	**0.65 (0.059, 1.24) p=0.0352**			
Trial 5	*B. suis*	Aluminum	**0.77 (0.60, 0.94) p=0.0002**	**1.15 (0.88, 1.41) p<0.0001**	**1.87 (1.32, 2.42) p=0.0005**		
		Carpet	**0.93 (0.11, 1.75) p=0.0312**	**4.24 (3.28, 5.20) p<0.0001**	**>5.00 (4/5) p=0.0079**		
		Keyboard	**1.10 (0.94, 1.26) p<0.0001**	**1.67 (1.06, 2.28) p=0.0027**	**2.26 (1.50, 3.03) p=0.0022**		
		Painted Joint Tape	**>4.41 (4/5) p=0.0079**	**>1.05 (1/5) p=0.3571**	**>3.33 (5/5) p=0.0079**		

Trials	Agent	Material	Mean Log Reduction (95% CI) and p-Value* or Mean Log Reduction (# of treated coupons with zero recovery/# of treated coupons) and p-Value† or N/A (# of control coupons with zero recovery/# of control coupons # of treated coupons with zero recovery/# of treated coupons) and p-Value‡				
			15 min	30 min	60 min	75 min	90 min
Trial 6	*Y. pestis*	Aluminum	**>4.72 (4/5) p=0.0079**	**>4.46 (5/5) p=0.0079**			
		Carpet	-0.49 (-2.17, 1.18) p=0.5198	**3.22 (1.27, 5.16) p=0.0187**			
		Keyboard	**>2.66 (1/5) p=0.0079**	**>4.33 (2/5) p=0.0079**			
		Painted Joint Tape	**>3.32 (3/5) p=0.0079**	**>2.39 (2/5) p=0.0079**			
Trial 7	*Y. pestis*	Aluminum	**2.18 (1.81, 2.54) p<0.0001**	**>2.17 (1/5) p=0.0079**	**>4.45 (3/5) p=0.0079**		
		Carpet	0.25 (-0.082, 0.57) p=0.1294	-0.056 (-0.54, 0.43) p=0.7987	0.14 (-0.056, 0.34) p=0.1406		
		Keyboard	**1.06 (0.41, 1.72) p=0.0062**	**1.51 (1.12, 1.90) p=0.0005**	**2.02 (1.62, 2.41) p=0.0002**		
		Painted Joint Tape	0.23 (-1.71, 2.16) p=0.7959	N/A (1/5 4/5) p=0.2857	**>2.03 (5/5) p=0.0079**		
Trial 8	*B. anthracis* spores	Carpet		**0.80 (0.63, 0.97) p=0.0002**	-0.38 (-2.77, 2.02) p=0.7344		
		Keyboard		**>6.12 (5/5) p=0.0079**	**>6.08 (5/5) p=0.0079**		
Trial 9a	*B. anthracis* spores	Aluminum				**0.81 (0.38, 1.24) p=0.0112**	
		Carpet				**>4.97 (5/5) p=0.0079**	
		Keyboard				**>6.22 (5/5) p=0.0079**	
		Painted Joint Tape				**>4.99 (5/5) p=0.0079**	

* Mean log reduction is the mean of the base-10 logarithm of recovered agent from the control coupons minus the mean of the base-10 logarithm of recovered agent from the treated coupons. A 95 % CI for the difference is shown in parentheses. A p-value is provided for the probability that the control and treatment recoveries are the same. The p-value is from the two sample t-test with Satterthwaite's method to allow for potentially different variances in the two groups. p-Values less than 0.05 denote less than 1 in 20 chance that a difference as large as or larger than observed would occur by chance if the control and treatment means were truly identical. Comparisons with p-values less than 0.05 (statistically significant at the 0.05 level) are bolded.

† One or more of the treatment coupons had no recovered agent. The mean log reduction of the form ">X" is calculated as the mean of the base-10 logarithm of recovered agent from the control coupons minus the mean of the base-10 logarithm of recovered agent from the treated coupons except that "zero recovery" coupons have a substituted recovered value of "1" (base-10 log is 0). Since the log becomes an increasingly negative value below 1 and is undefined at 0, this substitution is necessary and results in a lower bound on the mean log difference, as indicated by the ">". The number of "zero recovery" treatment coupons and the total number of treatment coupons is shown in parentheses. The p-value is from the non-parametric Kolmogorov-Smirnov test. p-Values less than 0.05 denote less than 1 in 20 chance results as different or more different than observed would occur by chance if the distribution of the control and treatment recoveries were truly identical. Comparisons with p-values less than 0.05 (statistically significant at the 0.05 level) are bolded.

‡ One or more of both the control and the treatment coupons had no recovered agent. In this case, the log reduction is indeterminate and the mean log reduction is identified as "N/A". The number of "zero recovery" control coupons and the total number of control coupons is shown in parentheses followed by the number of "zero recovery" treatment coupons and the total number of treatment coupons. The p-value is from the non-parametric Kolmogorov-Smirnov test. p-Values less than 0.05 denote less than 1 in 20 chance that results as different as or more different than observed would occur by chance if the distribution of the control and treatment recoveries were truly identical. Comparisons with p-values less than 0.05 (statistically significant at the 0.05 level) are bolded.

5.4 HP Fumigation (STERIS VHP®)

5.4.1 Description of STERIS VHP® HP Technology

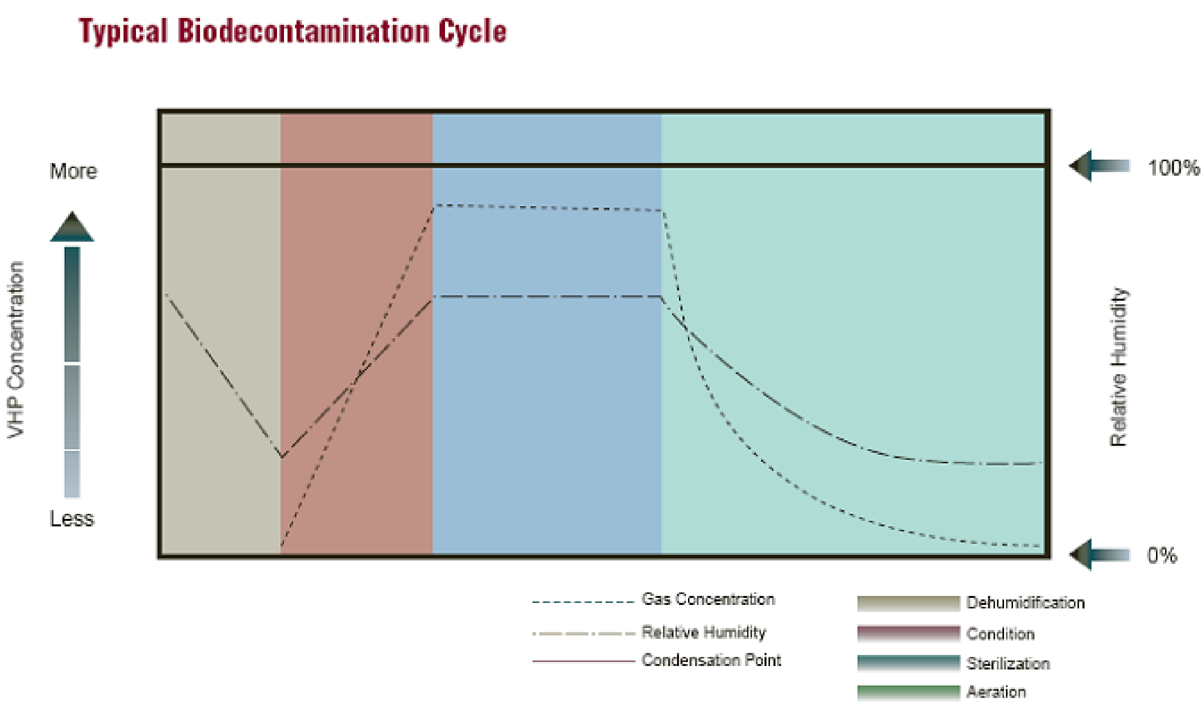
The STERIS VHP® Generator Series 1000ED, shown in Figure 5-16, was used to introduce and control the HP vapor inside a 1270 L BSC III. Because HP vapor is not stable as a compressed gas, HP vapor must be produced on site by vaporization of concentrated aqueous solutions of HP. Thus, this technology includes the equipment and chemicals for on-site generation, dispersion, and neutralization of the HP vapor.

Figure 5-16. STERIS VHP® Generator Series 1000ED.[16]

The HP fumigation technology operates at ambient temperature and atmospheric pressure in a closed loop configuration. As depicted in Figure 5-17 (from STERIS literature), the testing chamber is subjected to four phases: dehumidification, condition, sterilization, and aeration. This technology dehumidifies by re-circulating the chamber air through a reusable or disposable desiccant cartridge. Once the desired RH is reached, HP vapor is injected at a rate set to achieve the desired concentration of HP inside the chamber. The system then maintains the set concentration for the desired contact period for decontamination of the biological agent. Once the decontamination phase is complete, the enclosure air is re-circulated through the HP fumigation technology to reduce the HP vapor concentration to the desired level.

The HP vapor in the test chamber is monitored using an Analytical Technology HP gas sensor with liquid crystal display, as described in Section 5.2.1. The HP gas sensor is oriented in the test chamber at a position that is distant to the HP vaporizer but in proximity to test coupons. The display unit and power supply are located outside the test chamber. The concentration of HP vapor is documented approximately every 20 min during the gassing portion of the decontamination cycle while test coupons are in the test chamber.

Figure 5-17. STERIS VHP® Biodecontamination Cycle.[16]



5.4.2 Test Matrix for STERIS VHP® HP Fumigation

The testing performed with STERIS VHP® HP fumigation is shown in Table 5-26. The experimental design tested decontamination efficacy by determining whether there was a difference between the log reductions in the viable biological agents after fumigation compared to controls for various materials. These tests also assessed whether there was any difference in efficacy at varying fumigant concentrations and fumigation contact times. Critical parameters in this testing included fumigant concentration, fumigation contact time, and the viability of the biological agents. An adaptive management approach was used to incorporate new knowledge into the testing as decontamination efficacy results became available.

Table 5-26. Test Matrix for STERIS VHP® HP Fumigation

Trial	Biological Agent*	Material	STERIS VHP® Concentration	Contact Times (Sterilization Phase)
1	*B. anthracis* spores	Aluminum Keyboard Carpet Joint tape	500 ppmv	30 min, 60 min, 120 min, 240 min, and full cycle 240 min
4a	*Y. pestis*	Aluminum Keyboard	200-250 ppmv	90 min, 120 min
4b	*Y. pestis*	Aluminum Keyboard	500 ppmv	30 min
5a	*F. tularensis*	Aluminum Keyboard	200-250 ppmv	90 min
5b	*F. tularensis*	Aluminum Keyboard	500 ppmv	30 min
6a	*B. suis*	Aluminum Keyboard Carpet Joint tape	200-250 ppmv	90 min, 120 min
6b	*B. suis*	Aluminum Keyboard Carpet Joint tape	500 ppmv	30 min, 60 min, 90 min
7a	Vaccinia virus	Aluminum Keyboard Carpet Joint tape	200-250 ppmv	30 min, 60 min[†], 120 min[‡]
7b	Vaccinia virus	Aluminum Keyboard	500 ppmv	30 min, 60 min
2a	*B. anthracis* spores	Laminate Ductwork Carpet Concrete	500 ppmv	30 min, 60 min, 120 min, 240 min, and full cycle 240 min
2b	*B. anthracis* spores	Laminate Ductwork Carpet Concrete	200-250 ppmv	30 min, 60 min, 120 min, 240 min, and full cycle 240 min
3a	*B. anthracis* spores	Wood Glass Ceiling tile	500 ppmv	30 min, 60 min, 120 min, 240 min, and full cycle 240 min
3b	*B. anthracis* spores	Wood Glass Ceiling tile	200-250 ppmv	30 min, 60 min, 120 min, 240 min, and full cycle 240 min

* Biological indicators (*G. stearothermophilus*) were included with each fumigation test.
† At 60 min only aluminum and keyboard were used.
‡ At 120 min only aluminum was used.

5.4.3 STERIS VHP® HP Fumigation Results

Figure 5-18 shows a graph of typical temperature, RH, and HP concentration during a nominally 225 ppmv HP fumigation cycle.

Figure 5-18. Typical Temperature (˚C), RH (%), and HP Concentration (ppmv) Dynamics During a Fumigation Cycle (225 ppmv HP) with the STERIS VHP® Generator Series 1000ED.

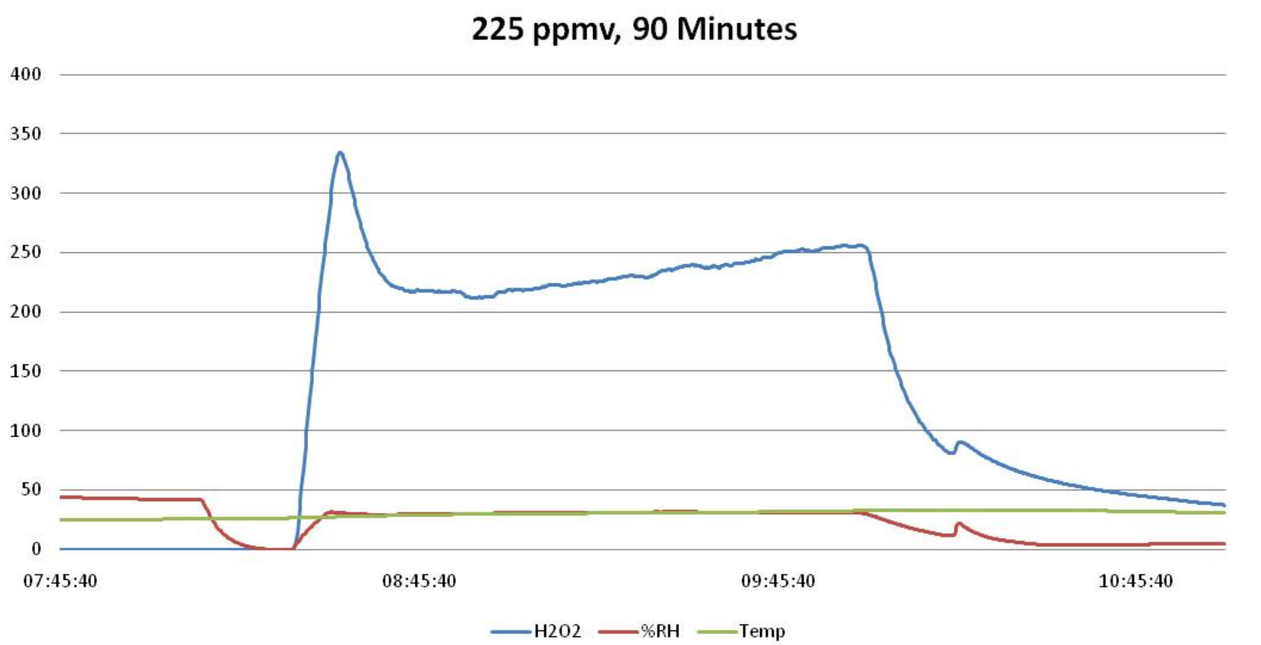
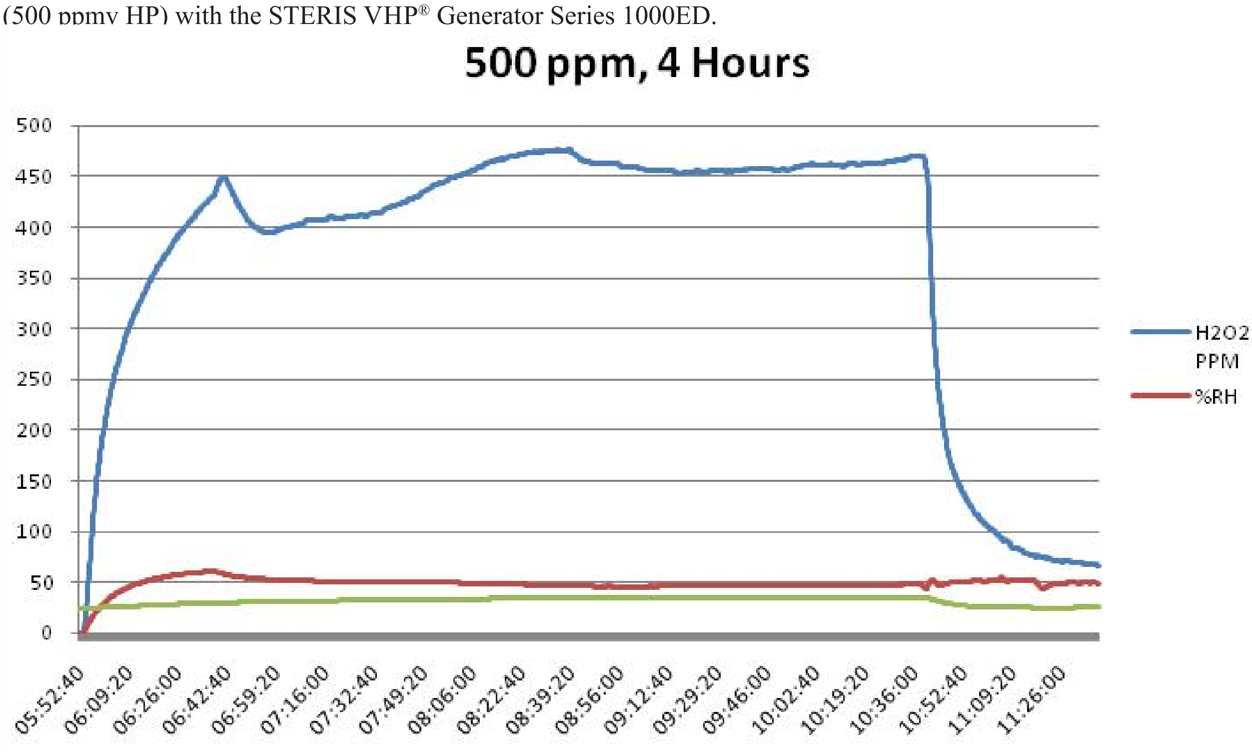


Figure 5-19 shows a graph of typical temperature, RH, and HP concentration during a nominally 500 ppmv HP fumigation cycle.

Figure 5-19. Typical Temperature (˚C), RH (%), and HP Concentration (ppmv) Dynamics During a Fumigation Cycle (500 ppmv HP) with the STERIS VHP® Generator Series 1000ED.



G. stearothermophilus

The results for STERIS VHP® HP fumigation of biological indicators are shown in Table 5-27. The biological indicators were *G. stearothermophilus* nominally 1 x 10^6 spores on stainless steel in Tyvek® packaging. In this qualitative test, no growth was observed at any contact time of 60 min or longer at an HP concentration of 500 ppmv; no growth was observed at any contact time of 240 min at an HP concentration of 200-250 ppmv. On some materials and with some biological agents (*B. anthracis*, *B. suis*, and vaccinia virus), the results from the biological indicators did not correlate with the results from the biological agents' exposure to the same CT. The high efficacy, suggested by the completed kill of the biological indicators, did not correlate with complete kill of *B. anthracis* spores on other materials.

Table 5-27. STERIS VHP® HP Fumigation Results for *G. stearothermophilus*

Trial	STERIS VHP® Concentration	Contact Times (min) Growth Positive/Total Biological Indicators			
1	500 ppmv	30 5/5	60 0/5	120 0/5	240 0/5
2a	500 ppmv	30 5/5	60 0/5	120 0/5	240 0/5
2b	200-250 ppmv	30 5/5	60 5/5	120 2/5	240 0/5
3a	500 ppmv	30 4/5	60 0/5	120 0/5	240 0/5
3b	200-250 ppmv	30 5/5	60 5/5	120 2/5	240 0/5
4a	200-250 ppmv		90 0/5	120 0/5	
4b	500 ppmv	30 1/5			
5a	200-250 ppmv		90 0/5		
5b	500 ppmv	30 1/5			
6a	200-250 ppmv		90 0/5	120 0/5	
6b	500 ppmv	30 1/5	60 0/5	90 0/5	
7a	200-250 ppmv	30 3/5	60 0/5	120 0/5	
7b	500 ppmv	30 0/5	60 0/5		

B. anthracis

STERIS VHP® HP fumigation results for *B. anthracis* spores are presented in Table 5-28 and Figures 5-20 and 5-21. No viable *B. anthracis* spores were recovered from carpet, concrete, glass, aluminum, keyboard, laminate, ductwork, and ceiling tile at exposures of 120 min to the 500 ppmv HP fumigation cycle. Decontamination of wood was more difficult than other materials: *B. anthracis* spores were recovered from wood after 120-min exposure to the 500 ppmv HP fumigation cycle. No viable *B. anthracis* spores were recovered from wood at an exposure of 240 min to the 500 ppmv HP fumigation cycle.

With the 200-250 ppmv HP fumigation cycle, *B. anthracis* spores were not recovered from laminate, ductwork, concrete, glass, and ceiling tile after 120 min of exposure, but *B. anthracis* spores were recovered from carpet and wood after the entire 240-min fumigation cycle. For perspective, Vaprox® HP sterilant, when used with a STERIS VHP® generator for sterilization of exposed pre-cleaned dry porous and non-porous surfaces, specifies a sterilization phase with "a minimum of 250 ppm[v] of VHP sterilant for 90 min in sealed enclosures up to 4,000 ft^3 [113,000 L]".[17]

Table 5-28. STERIS VHP® HP Fumigation Results for *B. anthracis*

Trial	Contact Time	Material	Spike Amount (CFU/coupon)	Mean Recovered *B. anthracis* (CFU/coupon)*		Mean Log Reduction*
				Positive Control†	Test Coupon‡	
500 ppmv Fumigation Cycle						
1	30 min	Aluminum	4.87 x 10^{8} §	7.30 ± 4.03 x 10^{8} ¶	0.00 ± 0.00	8.86 ± 0.00
		Keyboard	4.87 x 10^{8} §	3.96 ± 3.29 x 10^{8}	0.00 ± 0.00	8.60 ± 0.00
		Carpet – a	4.87 x 10^{8} §	7.70 ± 4.24 x 10^{8} ¶	2.73 ± 4.06 x 10^{5}	4.31 ± 1.21
		Joint tape	4.87 x 10^{8} §	5.42 ± 1.72 x 10^{8}	0.00 ± 0.00	8.73 ± 0.00
1	60 min	Aluminum	4.87 x 10^{8} §	7.30 ± 4.03 x 10^{8} ¶	0.00 ± 0.00	8.86 ± 0.00
		Keyboard	4.87 x 10^{8} §	3.96 ± 3.29 x 10^{8}	0.00 ± 0.00	8.60 ± 0.00
		Carpet – a	4.87 x 10^{8} §	7.70 ± 4.24 x 10^{8} ¶	1.85 ± 2.56 x 10^{4}	6.70 ± 2.35
		Joint tape	4.87 x 10^{8} §	5.42 ± 1.72 x 10^{8}	0.00 ± 0.00	8.73 ± 0.00
1	120 min	Aluminum	4.87 x 10^{8} §	7.30 ± 4.03 x 10^{8} ¶	0.00 ± 0.00	8.86 ± 0.00
		Keyboard	4.87 x 10^{8} §	3.96 ± 3.29 x 10^{8}	0.00 ± 0.00	8.60 ± 0.00
		Carpet – a	4.87 x 10^{8} §	7.70 ± 4.24 x 10^{8} ¶	0.00 ± 0.00	8.89 ± 0.00
		Joint tape	4.87 x 10^{8} §	5.42 ± 1.72 x 10^{8}	0.00 ± 0.00	8.73 ± 0.00
1	240 min	Aluminum	4.87 x 10^{8} §	7.30 ± 4.03 x 10^{8} ¶	0.00 ± 0.00	8.86 ± 0.00
		Keyboard	4.87 x 10^{8} §	3.96 ± 3.29 x 10^{8}	0.00 ± 0.00	8.60 ± 0.00
		Carpet – a	4.87 x 10^{8} §	7.70 ± 4.24 x 10^{8} ¶	0.00 ± 0.00	8.89 ± 0.00
		Joint tape	4.87 x 10^{8} §	5.42 ± 1.72 x 10^{8}	0.00 ± 0.00	8.73 ± 0.00
1	Full cycle 240 min	Aluminum	4.87 x 10^{8} §	7.30 ± 4.03 x 10^{8} ¶	0.00 ± 0.00	8.86 ± 0.00
		Keyboard	4.87 x 10^{8} §	3.96 ± 3.29 x 10^{8}	0.00 ± 0.00	8.60 ± 0.00
		Carpet	4.87 x 10^{8} §	7.70 ± 4.24 x 10^{8} ¶	0.00 ± 0.00	8.89 ± 0.00
		Joint tape	4.87 x 10^{8} §	5.42 ± 1.72 x 10^{8}	0.00 ± 0.00	8.73 ± 0.00
500 ppmv Fumigation Cycle						
2a	30 min	Laminate	2.80 x 10^{8} §	2.61 ± 1.58 x 10^{8}	0.00 ± 0.00	8.42 ± 0.00
		Ductwork	2.80 x 10^{8} §	1.67 ± 1.04 x 10^{8}	0.00 ± 0.00	8.22 ± 0.00
		Carpet – b	2.80 x 10^{8} §	2.13 ± 2.39 x 10^{8}	1.74 ± 1.41 x 10^{5}	3.44 ± 0.85
		Concrete	2.80 x 10^{8} §	2.26 ± 1.60 x 10^{8}	1.20 ± 2.68 x 10^{2}	7.80 ± 1.24
2a	60 min	Laminate	2.80 x 10^{8} §	2.61 ± 1.58 x 10^{8}	0.00 ± 0.00	8.42 ± 0.00
		Ductwork	2.80 x 10^{8} §	1.67 ± 1.04 x 10^{8}	0.00 ± 0.00	8.22 ± 0.00
		Carpet – b	2.80 x 10^{8} §	2.13 ± 2.39 x 10^{8}	6.67 ± 11.6 x 10^{3}	5.57 ± 1.69
		Concrete	2.80 x 10^{8} §	2.26 ± 1.60 x 10^{8}	0.00 ± 0.00	8.35 ± 0.00
2a	120 min	Laminate	2.80 x 10^{8} §	2.61 ± 1.58 x 10^{8}	0.00 ± 0.00	8.42 ± 0.00
		Ductwork	2.80 x 10^{8} §	1.67 ± 1.04 x 10^{8}	0.00 ± 0.00	8.22 ± 0.00
		Carpet – b	2.80 x 10^{8} §	2.13 ± 2.39 x 10^{8}	0.00 ± 0.00	8.33 ± 0.00
		Concrete	2.80 x 10^{8} §	2.26 ± 1.60 x 10^{8}	0.00 ± 0.00	8.35 ± 0.00
2a	240 min	Laminate	2.80 x 10^{8} §	2.61 ± 1.58 x 10^{8}	0.00 ± 0.00	8.42 ± 0.00
		Ductwork	2.80 x 10^{8} §	1.67 ± 1.04 x 10^{8}	0.00 ± 0.00	8.22 ± 0.00
		Carpet – b	2.80 x 10^{8} §	2.13 ± 2.39 x 10^{8}	0.00 ± 0.00	8.33 ± 0.00
		Concrete	2.80 x 10^{8} §	2.26 ± 1.60 x 10^{8}	0.00 ± 0.00	8.35 ± 0.00
2a	Full cycle 240 min	Laminate	2.80 x 10^{8} §	2.61 ± 1.58 x 10^{8}	0.00 ± 0.00	8.42 ± 0.00
		Ductwork	2.80 x 10^{8} §	1.67 ± 1.04 x 10^{8}	0.00 ± 0.00	8.22 ± 0.00
		Carpet	2.80 x 10^{8} §	2.13 ± 2.39 x 10^{8}	0.00 ± 0.00	8.33 ± 0.00
		Concrete	2.80 x 10^{8} §	2.26 ± 1.60 x 10^{8}	0.00 ± 0.00	8.35 ± 0.00

Trial	Contact Time	Material	Spike Amount (CFU/ coupon)	Mean Recovered *B. anthracis* (CFU/coupon)*		Mean Log Reduction*
				Positive Control†	Test Coupon‡	
200-250 ppmv Fumigation Cycle						
2b	30 min	Laminate	6.93 x 10^6 #	3.22 ± 0.50 x 10^6	1.34 ± 3.00 x 10^1	6.14 ± 0.82
		Ductwork	6.93 x 10^6 #	5.25 ± 0.79 x 10^6	0.00 ± 0.00	6.72 ± 0.00
		Carpet	6.93 x 10^6 #	6.17 ± 0.57 x 10^6	3.35 ± 0.74 x 10^5	1.27 ± 0.09
		Concrete	6.93 x 10^6 #	6.73 ± 0.70 x 10^6	1.65 ± 3.59 x 10^3	5.28 ± 1.63
2b	60 min	Laminate	6.93 x 10^6 #	3.22 ± 0.50 x 10^6	0.00 ± 0.00	6.51 ± 0.00
		Ductwork	6.93 x 10^6 #	5.25 ± 0.79 x 10^6	0.00 ± 0.00	6.72 ± 0.00
		Carpet	6.93 x 10^6 #	6.17 ± 0.57 x 10^6	1.11 ± 2.01 x 10^3	5.06 ± 1.67
		Concrete	6.93 x 10^6 #	6.73 ± 0.70 x 10^6	6.60 ± 14.8 x 10^0	6.52 ± 0.68
2b	120 min	Laminate	6.93 x 10^6 #	3.22 ± 0.50 x 10^6	0.00 ± 0.00	6.51 ± 0.00
		Ductwork	6.93 x 10^6 #	5.25 ± 0.79 x 10^6	0.00 ± 0.00	6.72 ± 0.00
		Carpet	6.93 x 10^6 #	6.17 ± 0.57 x 10^6	2.00 ± 4.47 x 10^1	6.39 ± 0.89
		Concrete	6.93 x 10^6 #	6.73 ± 0.70 x 10^6	0.00 ± 0.00	6.83 ± 0.00
2b	240 min	Laminate	6.93 x 10^6 #	3.22 ± 0.50 x 10^6	0.00 ± 0.00	6.51 ± 0.00
		Ductwork	6.93 x 10^6 #	5.25 ± 0.79 x 10^6	0.00 ± 0.00	6.72 ± 0.00
		Carpet	6.93 x 10^6 #	6.17 ± 0.57 x 10^6	2.00 ± 2.99 x 10^1	6.12 ± 0.92
		Concrete	6.93 x 10^6 #	6.73 ± 0.70 x 10^6	0.00 ± 0.00	6.83 ± 0.00
2b	Full cycle 240 min	Laminate	6.93 x 10^6 #	3.22 ± 0.50 x 10^6	0.00 ± 0.00	6.51 ± 0.00
		Ductwork	6.93 x 10^6 #	5.25 ± 0.79 x 10^6	0.00 ± 0.00	6.72 ± 0.00
		Carpet	6.93 x 10^6 #	6.17 ± 0.57 x 10^6	1.34 ± 3.00^1	6.42 ± 0.82
		Concrete	6.93 x 10^6 #	6.73 ± 0.70 x 10^6	0.00 ± 0.00	6.83 ± 0.00
500 ppmv Fumigation Cycle						
3a	30 min	Wood	9.77 x 10^6	5.47 ± 1.57 x 10^5 ¦	1.82 ± 3.23 x 10^4	1.95 ± 0.66
		Glass	9.77 x 10^6	8.18 ± 10.5 x 10^6	2.73 ± 3.52 x 10^2	5.40 ± 1.42
		Ceiling tile	9.77 x 10^6	7.49 ± 1.40 x 10^5 ¦	0.00 ± 0.00	5.87 ± 0.00
3a	60 min	Wood	9.77 x 10^6	5.47 ± 1.57 x 10^5 ¦	4.51 ± 4.41 x 10^3	2.97 ± 1.69
		Glass	9.77 x 10^6	8.18 ± 10.5 x 10^6	0.00 ± 0.00	6.91 ± 0.00
		Ceiling tile	9.77 x 10^6	7.49 ± 1.40 x 10^5 ¦	0.00 ± 0.00	5.87 ± 0.00
3a	120 min	Wood	9.77 x 10^6	5.47 ± 1.57 x 10^5 ¦	7.19 ± 8.28 x 10^2	3.51 ± 1.31
		Glass	9.77 x 10^6	8.18 ± 10.5 x 10^6	0.00 ± 0.00	6.91 ± 0.00
		Ceiling tile	9.77 x 10^6	7.49 ± 1.40 x 10^5 ¦	0.00 ± 0.00	5.87 ± 0.00
3a	240 min	Wood	9.77 x 10^6	5.47 ± 1.57 x 10^5 ¦	0.00 ± 0.00	5.74 ± 0.00
		Glass	9.77 x 10^6	8.18 ± 10.5 x 10^6	0.00 ± 0.00	6.91 ± 0.00
		Ceiling tile	9.77 x 10^6	7.49 ± 1.40 x 10^5 ¦	0.00 ± 0.00	5.87 ± 0.00
3a	Full cycle 240 min	Wood	9.77 x 10^6	5.47 ± 1.57 x 10^5 ¦	0.00 ± 0.00	5.74 ± 0.00
		Glass	9.77 x 10^6	8.18 ± 10.5 x 10^6	0.00 ± 0.00	6.91 ± 0.00
		Ceiling tile	9.77 x 10^6	7.49 ± 1.40 x 10^5 ¦	0.00 ± 0.00	5.87 ± 0.00

Trial	Contact Time	Material	Spike Amount (CFU/ coupon)	Mean Recovered *B. anthracis* (CFU/coupon)*		Mean Log Reduction*
				Positive Control†	Test Coupon‡	
200-250 ppmv Fumigation Cycle						
3b	30 min	Wood	1.02 x 10^7	3.59 ± 0.63 x 10^5¦	2.06 ± 1.60 x 10^4	1.35 ± 0.35
		Glass	1.02 x 10^7	3.53 ± 1.68 x 10^6	1.34 ± 3.00 x 10^1	6.18 ± 0.82
		Ceiling tile	1.02 x 10^7	7.62 ± 2.18 x 10^5¦	3.32 ± 4.08 x 10^1	5.18 ± 0.98
3b	60 min	Wood	1.02 x 10^7	3.59 ± 0.63 x 10^5¦	6.83 ± 10.9 x 10^3	2.15 ± 0.70
		Glass	1.02 x 10^7	3.53 ± 1.68 x 10^6	2.66 ± 5.95 x 10^1	6.12 ± 0.95
		Ceiling tile	1.02 x 10^7	7.62 ± 2.18 x 10^5¦	2.00 ± 2.99 x 10^1	5.21 ± 0.92
3b	120 min	Wood	1.02 x 10^7	3.59 ± 0.63 x 10^5¦	2.68 ± 0.60 x 10^3	2.14 ± 0.10
		Glass	1.02 x 10^7	3.53 ± 1.68 x 10^6	0.00 ± 0.00	6.55 ± 0.00
		Ceiling tile	1.02 x 10^7	7.62 ± 2.18 x 10^5¦	0.00 ± 0.00	5.88 ± 0.00
3b	240 min	Wood	1.02 x 10^7	3.59 ± 0.63 x 10^5¦	1.61 ± 1.67 x 10^3	3.52 ± 1.87
		Glass	1.02 x 10^7	3.53 ± 1.68 x 10^6	0.00 ± 0.00	6.55 ± 0.00
		Ceiling tile	1.02 x 10^7	7.62 ± 2.18 x 10^5¦	0.00 ± 0.00	5.88 ± 0.00
3b	Full cycle 240 min	Wood	1.02 x 10^7	3.59 ± 0.63 x 10^5¦	2.73 ± 3.84 x 10^4	3.32 ± 2.45
		Glass	1.02 x 10^7	3.53 ± 1.68 x 10^6	0.00 ± 0.00	6.55 ± 0.00
		Ceiling tile	1.02 x 10^7	7.62 ± 2.18 x 10^5¦	0.00 ± 0.00	5.88 ± 0.00

* Data are expressed as mean ± standard deviation of five replicates.
† Positive control coupons were spiked but not exposed to the fumigant.
‡ Test coupons were spiked and exposed to the fumigant for the contact time.
¦ Time zero was lower than target recovery of ≥10% of spike amount.
¶ Time zero exceeds target recovery of ≤120% of spike amount.
§ Application was inadvertently about 1 log higher than the target 1 x 10^7 CFUs/coupon.
# Application was lower than the target 7.5 x 10^5 CFUs/coupon.

Figure 5-20. STERIS VHP® Fumigation Results for *B. anthracis* at the 500 ppmv HP Fumigation Cycle, Line Chart.

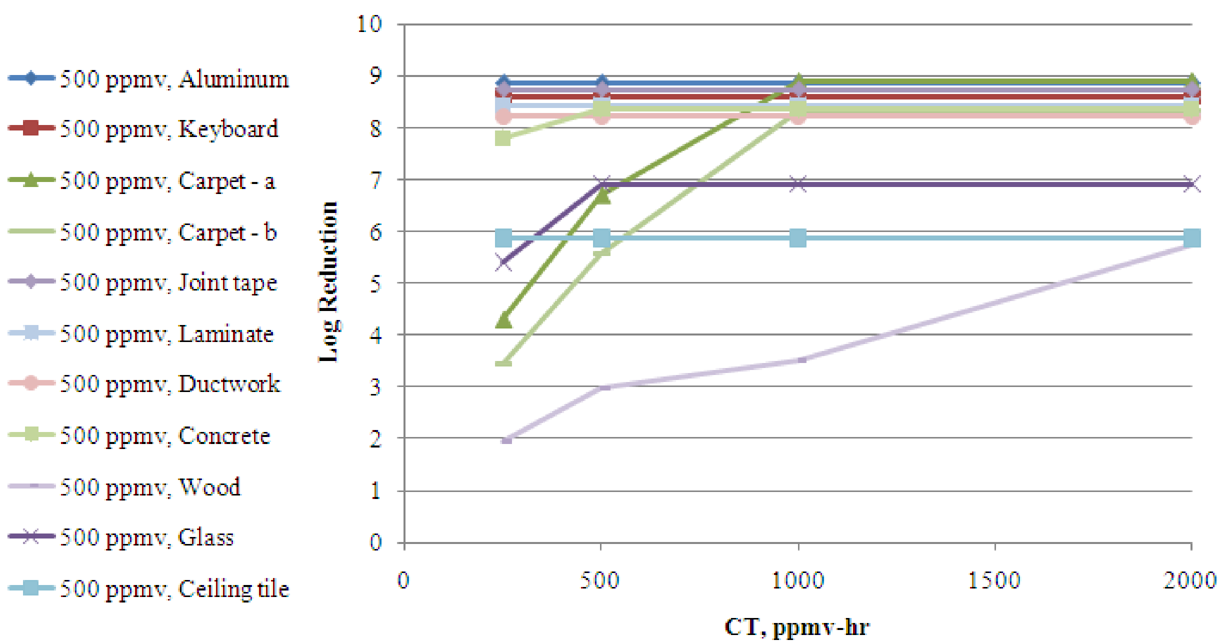
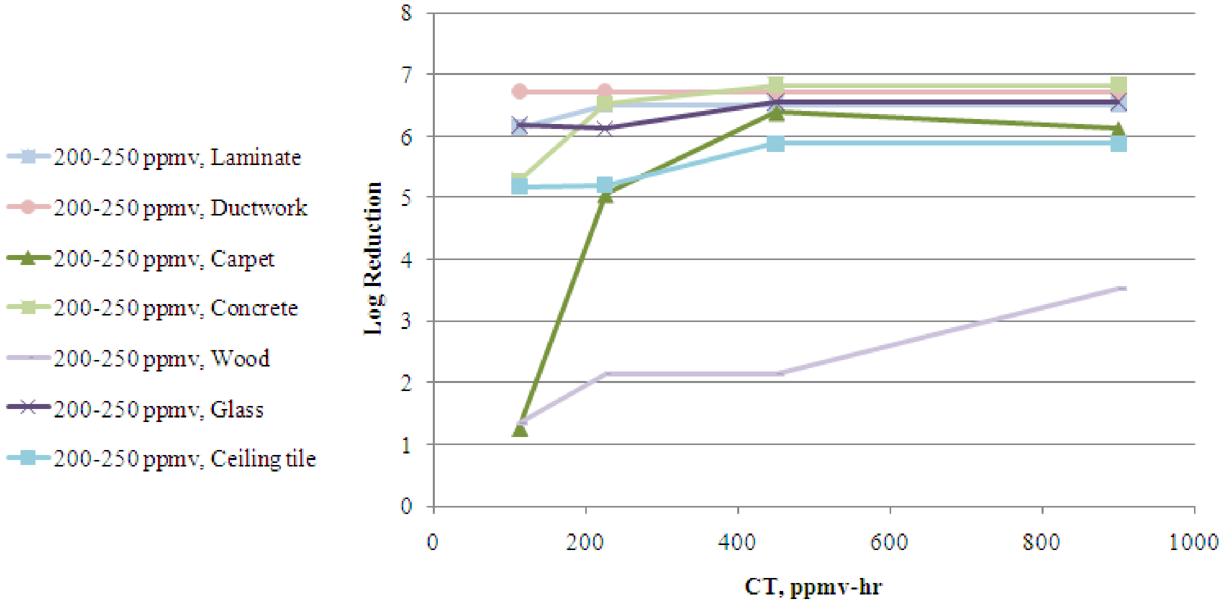


Figure 5-21. STERIS VHP® Fumigation Results for *B. anthracis* at the 200-250 ppmv HP Fumigation Cycle, Line Chart.



B. suis

STERIS VHP® HP fumigation results for *B. suis* are presented in Table 5-29 and Figures 5-22 and 5-23. After exposure to the 500 ppmv fumigation cycle, *B. suis* was not recovered from any material after a 90-min exposure. For carpet and joint tape shorter exposures (30 min) resulted in no recoveries of viable *B. suis*. After a 120-min exposure to the 200-250 ppmv HP fumigation cycle, viable *B. suis* was recovered at low levels (~10^0 to ~10^1 CFUs/coupon) from keyboard and joint tape; no viable *B. suis* was recovered from aluminum and carpet.

Table 5-29. STERIS VHP® HP Fumigation Results for *B. suis*

Trial	Contact Time	Material	Spike Amount (CFU/ coupon)	Mean Recovered *B. suis* (CFU/coupon)*		Mean Log Reduction*
				Positive Control†	Test Coupon‡	
200-250 ppmv Fumigation Cycle						
6a	90 min	Aluminum	6.83 x 10^7	5.11 ± 0.89 x 10^7	7.98 ± 8.37 x 10^1	6.22 ± 0.91
		Keyboard	6.83 x 10^7	4.99 ± 0.18 x 10^7	1.77 ± 1.84 x 10^3	5.03 ± 1.06
		Carpet	6.83 x 10^7	9.41 ± 2.64 x 10^4	0.00 ± 0.00	4.97 ± 0.00
		Joint tape	6.83 x 10^7	5.06 ± 3.48 x 10^5	0.00 ± 0.00	5.70 ± 0.00
6a	120 min	Aluminum	9.57 x 10^7	3.00 ± 0.74 x 10^7	0.00 ± 0.00	7.48 ± 0.00
		Keyboard	9.57 x 10^7	1.93 ± 1.51 x 10^7	6.66 ± 14.9 x 10^1	6.78 ± 1.13
		Carpet	9.57 x 10^7	1.99 ± 0.39 x 10^6	0.00 ± 0.00	6.30 ± 0.00
		Joint tape	9.57 x 10^7	2.25 ± 0.13 x 10^6	6.60 ± 14.8 x 10^0	6.04 ± 0.70
500 ppmv Fumigation Cycle						
6b	30 min	Aluminum	5.33 x 10^7	4.07 ± 0.29 x 10^7	2.16 ± 0.68 x 10^3	4.29 ± 0.13
		Keyboard	5.33 x 10^7	3.79 ± 0.50 x 10^7	1.75 ± 0.42 x 10^3	4.35 ± 0.12
		Carpet	5.33 x 10^7	3.13 ± 0.49 x 10^6	0.00 ± 0.00	6.50 ± 0.00
		Joint tape	5.33 x 10^7	1.98 ± 1.13 x 10^4	0.00 ± 0.00	4.30 ± 0.00
6b	60 min	Aluminum	7.03 x 10^7	3.76 ± 1.62 x 10^7	2.40 ± 2.73 x 10^2	5.76 ± 1.11
		Keyboard	7.03 x 10^7	3.32 ± 0.51 x 10^7	8.68 ± 9.31 x 10^1	6.25 ± 1.17
		Carpet	7.03 x 10^7	7.43 ± 3.65 x 10^5	0.00 ± 0.00	5.87 ± 0.00
		Joint tape	7.03 x 10^7	4.02 ± 2.01 x 10^4	0.00 ± 0.00	4.60 ± 0.00
6b	90 min	Aluminum	8.80 x 10^7	5.06 ± 0.59 x 10^7	0.00 ± 0.00	7.70 ± 0.00
		Keyboard	8.80 x 10^7	5.01 ± 1.05 x 10^7	0.00 ± 0.00	7.70 ± 0.00

* Data are expressed as mean ± standard deviation of five replicates.
† Positive control coupons were spiked but not exposed to the fumigant.
‡ Test coupons were spiked and exposed to the fumigant for the contact time.

Figure 5-22. STERIS VHP® Fumigation Results for *B. suis* at the 200-250 ppmv and 500 ppmv HP Fumigation cycles, Line Chart.

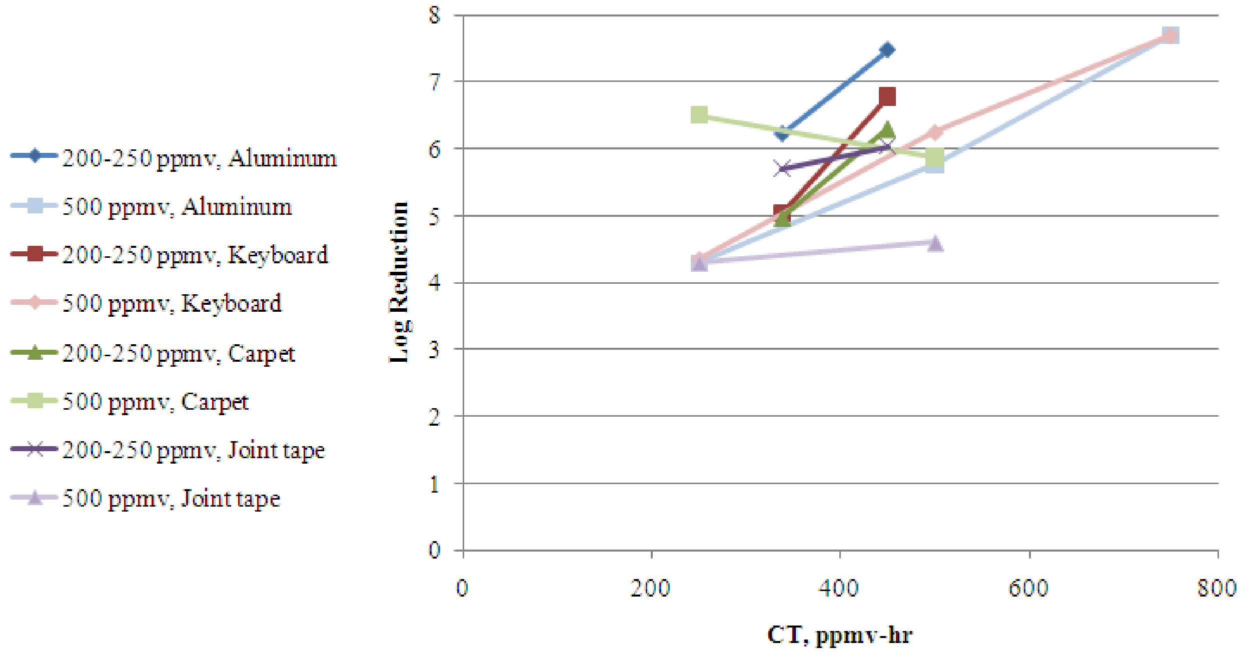
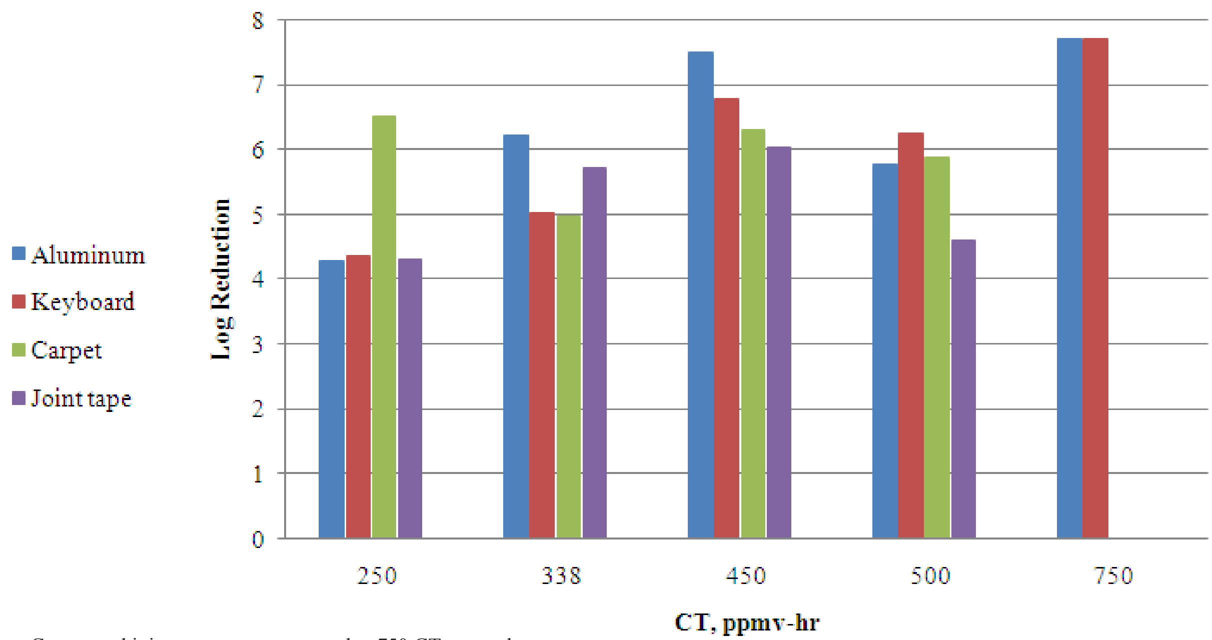


Figure 5-23. STERIS VHP® Fumigation Results for *B. suis* at the 200-250 ppmv and 500 ppmv HP Fumigation Cycles, Column Chart.



*Note: Carpet and joint tape were not tested at 750 CT, ppmv-hr.

F. tularensis

STERIS VHP® HP fumigation results for *F. tularensis* are presented in Table 5-30. Viable *F. tularensis* was not recovered after any of the fumigation trials; the associated mean log reductions in *F. tularensis* ranged from 5.59 to 6.66. Compared to the amount of bacteria spiked onto the coupon (about 10^8 CFUs/coupon), after a 90-min treatment of *F. tularensis* on aluminum and keyboard, a >8 log reduction in viable bacteria was attributable to the fumigation treatment and the loss of viability arising from other (unknown) time-dependent causes.

Table 5-30. STERIS VHP® HP Fumigation Results for *F. tularensis*

Trial	Contact Time	Material	Spike Amount (CFU/ coupon)	Mean Recovered *F. tularensis* (CFU/coupon)*		Mean Log Reduction*
				Positive Control†	Test Coupon‡	
200-250 ppmv Fumigation Cycle						
5a	90 min	Aluminum	1.91×10^{8}§	$2.18 \pm 0.91 \times 10^6$	0.00 ± 0.00	6.34 ± 0.00
		Keyboard	1.91×10^{8}§	$5.55 \pm 1.57 \times 10^5$	0.00 ± 0.00	5.74 ± 0.00
500 ppmv Fumigation Cycle						
5b	30 min	Aluminum	4.70×10^{8}§	$4.54 \pm 1.83 \times 10^6$	0.00 ± 0.00	6.66 ± 0.00
		Keyboard	4.70×10^{8}§	$3.87 \pm 1.10 \times 10^5$	0.00 ± 0.00	5.59 ± 0.00

* Data are expressed as mean ± standard deviation of five replicates.
† Positive control coupons were spiked but not exposed to the fumigant.
‡ Test coupons were spiked and exposed to the fumigant for the contact time.
§ Application was lower than the target 1.0 x 106 - 1.0 x 108 CFU/coupon.

Vaccinia virus

STERIS VHP® HP fumigation results for vaccinia virus are presented in Table 5-31 and Figures 5-24 and 5-25. No vaccinia virus was recovered from carpet or joint tape following exposure to the 200-250 ppmv HP fumigation cycle for 30 min. Vaccinia virus was recovered from keyboard after exposure to both the 200-250 ppmv HP and 500 ppmv HP fumigation cycles for 30 min, but vaccinia virus was not recovered from either fumigation cycle when the exposures lasted 60 min. Vaccinia virus was recovered from aluminum after every fumigation trial at levels <5 x 10^1 PFUs/coupon; the associated log reductions ranged from 4.60 to 5.26.

At the 200-250 ppmv fumigation cycle, growth was observed on three of five biological indicators following exposure to the fumigant after the 30-min contact time. At the 500 ppmv fumigation cycle, no growth was observed on any biological indicator replicate following exposure to the fumigant.

Compared to the amount of vaccinia virus spiked onto the coupon (about 10^7 PFUs/coupon), no viable vaccinia virus was recovered from carpet or joint tape after a treatment of 200-250 ppmv HP for 30-min contact time, equating to about a 7 log reduction in viable bacteria attributable to the fumigation and the loss of viability arising from other (unknown) time-dependent causes. Compared to the amount of vaccinia virus spiked onto the coupon (about 10^7 PFUs/coupon), no viable vaccinia virus was recovered from keyboard after a treatment of 200-250 ppmv HP for 60-min contact time, equating to about a 7 log reduction in viable vaccinia virus attributable to the combined effects of the fumigation and the loss of viability arising from other (unknown) time-dependent causes. Compared to the amount of vaccinia virus spiked onto the coupon (about 10^7 PFUs/coupon), no viable vaccinia was recovered from keyboard after a 200-250 ppmv HP fumigation for 60-min contact time or after a 500 ppmv HP fumigation with a 60-min contact time, equating to about a 7 log reduction in viable bacteria attributable to the combined effects of the fumigation and the loss of viability arising from other (unknown) time-dependent causes.

Table 5-31. STERIS VHP® HP Fumigation Results for Vaccinia Virus

Trial	Contact Time	Material	Spike Amount (PFU/coupon)	Mean Recovered Vaccinia Virus (PFU/coupon)*		Mean Log Reduction*
				Positive Control†	Test Coupon‡	
200-250 ppmv Fumigation Cycle						
7a	30 min	Aluminum	9.64×10^6	$2.50 \pm 1.59 \times 10^6$	$1.42 \pm 0.32 \times 10^1$	5.26 ± 0.11
		Keyboard	9.64×10^6	$2.43 \pm 0.60 \times 10^5$	$1.49 \pm 0.48 \times 10^1$	4.23 ± 0.16
		Carpet	9.64×10^6	$1.34 \pm 1.79 \times 10^4$	0.00 ± 0.00	4.13 ± 0.00
		Joint tape	9.64×10^6	$1.75 \pm 0.65 \times 10^4$	0.00 ± 0.00	4.24 ± 0.00
7a	60 min	Aluminum	5.99×10^6	$1.41 \pm 0.22 \times 10^6$	$1.25 \pm 0.21 \times 10^1$	5.06 ± 0.08
		Keyboard	5.99×10^6	$8.50 \pm 1.37 \times 10^4$	0.00 ± 0.00	4.93 ± 0.00
7a	120 min	Aluminum	6.51×10^6	$1.64 \pm 0.42 \times 10^6$	$3.37 \pm 0.67 \times 10^1$	4.70 ± 0.09
500 ppmv Fumigation Cycle						
7b	30 min	Aluminum	4.95×10^6	$1.28 \pm 0.68 \times 10^6$	$3.43 \pm 1.28 \times 10^1$	4.60 ± 0.19
		Keyboard	4.95×10^6	$2.58 \pm 0.66 \times 10^5$	$1.08 \pm 0.28 \times 10^1$	4.39 ± 0.12
7b	60 min	Aluminum	1.12×10^7	$2.27 \pm 0.17 \times 10^6$	$2.23 \pm 0.23 \times 10^1$	5.01 ± 0.05
		Keyboard	1.12×10^7	$4.78 \pm 1.37 \times 10^5$	0.00 ± 0.00	5.68 ± 0.00

* Data are expressed as mean ± standard deviation of five replicates.
† Positive control coupons were spiked but not exposed to the fumigant.
‡ Test coupons were spiked and exposed to the fumigant for the contact time.

Figure 5-24. STERIS VHP® Fumigation Results for Vaccinia Virus at the 200-250 ppmv and 500 ppmv HP Fumigation Cycles, Line Chart.

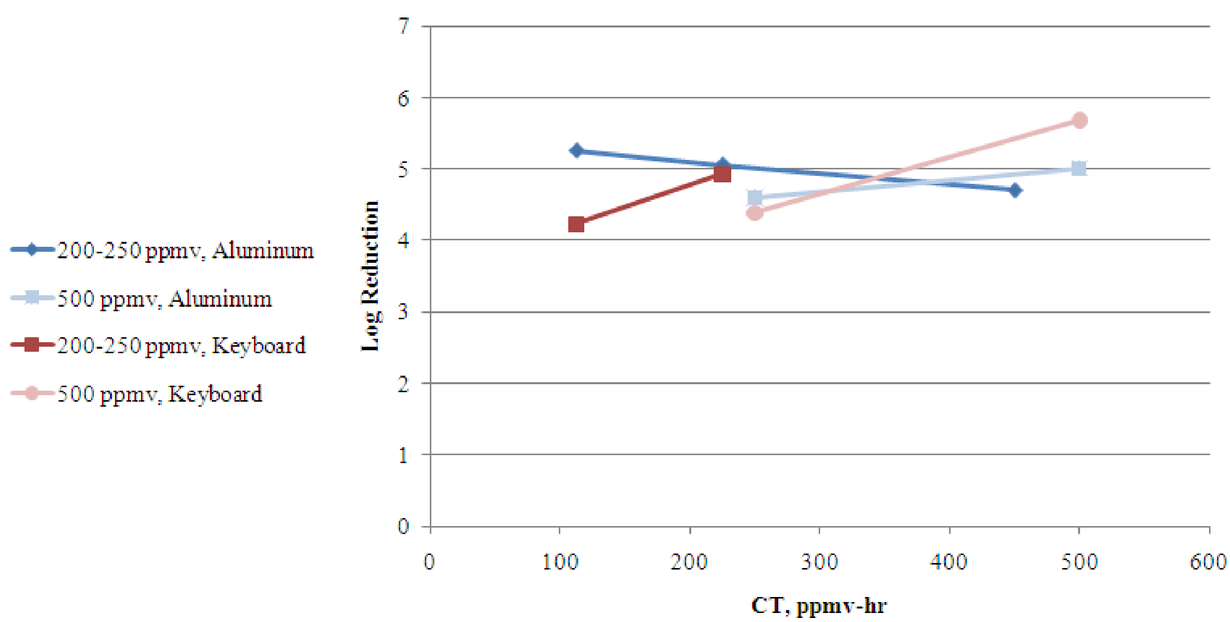
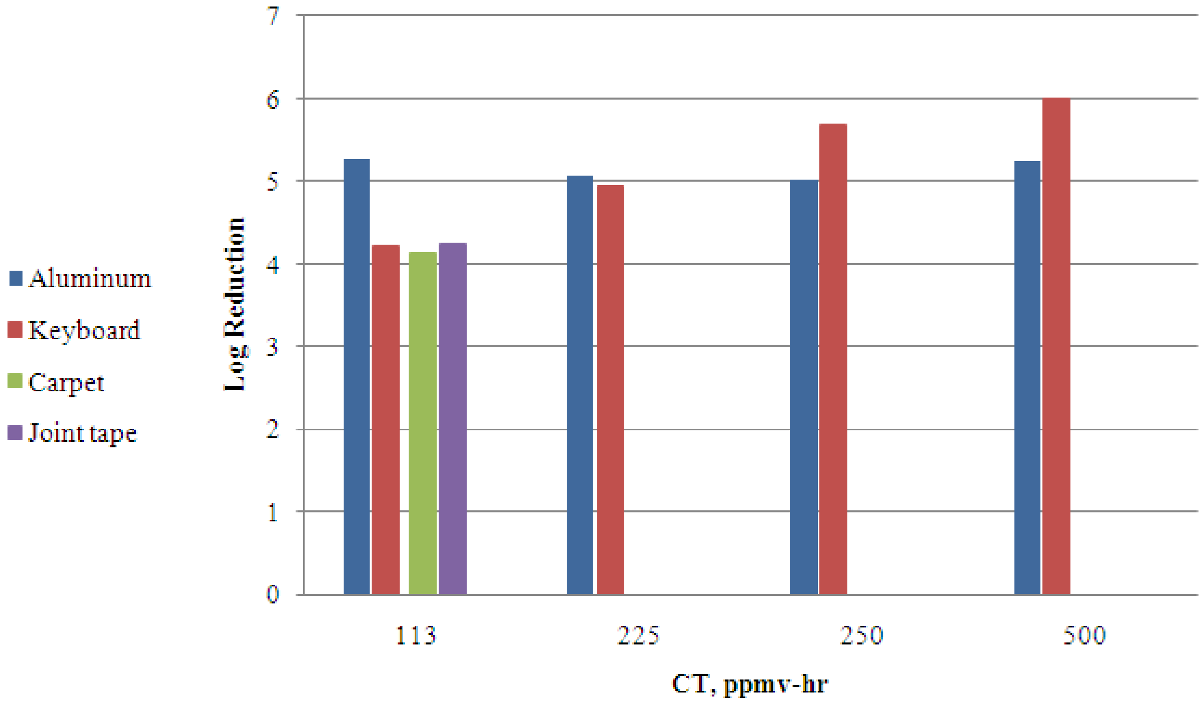


Figure 5-25. STERIS VHP® Fumigation Results for Vaccinia Virus at the 200-250 ppmv and 500 ppmv HP Fumigation Cycles, Column Chart.



Y. pestis

STERIS VHP® HP fumigation results for *Y. pestis* are presented in Table 5-32. Viable *Y. pestis* was not recovered after any of the fumigation trials; the associated mean log reductions in *Y. pestis* ranged from 1.90 to 4.73. Compared to the minimum amount of bacteria spiked onto the coupon (3.83 x 10^6 CFUs/coupon), after a 30-min treatment of *Y. pestis* on aluminum and keyboard, a >6.5 log reduction in viable bacteria was attributable to the fumigation treatment and the loss of viability arising from other (unknown) time-dependent causes.

Table 5-32. STERIS VHP® HP Fumigation Results for *Y. pestis*

Trial	Contact Time	Material	Spike Amount (CFU/coupon)	Mean Recovered *Y. pestis* (CFU/coupon)*		Mean Log Reduction*
				Positive Control†	Test Coupon‡	
200-250 ppmv Fumigation Cycle						
4a	90 min	Aluminum	3.83 x 10^6	3.29 ± 0.52 x 10^4	0.00 ± 0.00	4.52 ± 0.00
		Keyboard	3.83 x 10^6	4.13 ± 1.57 x 10^2	0.00 ± 0.00	2.62 ± 0.00
4a	120 min	Aluminum	3.57 x 10^6	5.31 ± 3.46 x 10^4	0.00 ± 0.00	4.73 ± 0.00
		Keyboard	3.57 x 10^6	8.00 ± 6.92 x 10^1	0.00 ± 0.00	1.90 ± 0.00
500 ppmv Fumigation Cycle						
4b	30 min	Aluminum	7.33 x 10^6	2.87 ± 1.70 x 10^4	0.00 ± 0.00	4.46 ± 0.00
		Keyboard	7.33 x 10^6	4.91 ± 0.73 x 10^4	0.00 ± 0.00	4.69 ± 0.00

* Data are expressed as mean ± standard deviation of five replicates.
† Positive control coupons were spiked but not exposed to the fumigant.
‡ Test coupons were spiked and exposed to the fumigant for the contact time.

Summary Statistics for STERIS VHP® HP Decontamination

Table 5-33 provides a summary of STERIS VHP® HP decontamination efficacy, calculated as the difference in the mean log of viable bacteria recovered from positive control coupons and the mean log of viable bacteria recovered from coupons after fumigation for a given contact time. The 95% CI and p-value are also shown. Significant log reduction was observed against all combinations of biological agents and materials at all contact times tested.

Surface Damage

The physical effect of the STERIS VHP® HP fumigation on the materials was evaluated qualitatively. The appearance of the decontaminated coupons was visually inspected for any obvious changes in the color, reflectivity, and apparent roughness of the material surfaces. These comparisons were performed for each material, before extraction of the decontaminated test coupons. No physical differences were observed between control and fumigated coupons for any material.

Table 5-33. Summary of STERIS VHP® HP Fumigation Efficacy (Calculated as Mean Log Reduction)

Trials	Agent	Material	Mean Log Reduction (95% CI) and P-value* Or Mean Log Reduction (# of treated coupons with zero recovery/# of treated coupons) and P-value†					
			30 min	**60 min**	**90 min**	**120 min**	**240 min**	**Gassing + 4hr**
Trial 1 (500 ppmv)	*B. anthracis* spores	Aluminum	**>8.70 (5/5) p=0.0079**	**>8.70 (5/5) p=0.0079**		**>8.70 (5/5) p=0.0079**	**>8.70 (5/5) p=0.0079**	**>8.70 (5/5) p=0.0079**
		Carpet	**4.17 (2.82, 5.51) p=0.0007**	**>6.57 (2/5) p=0.0079**		**>8.75 (5/5) p=0.0079**	**>8.75 (5/5) p=0.0079**	**>8.75 (5/5) p=0.0079**
		Keyboard	**>8.35 (5/5) p=0.0079**	**>8.35 (5/5) p=0.0079**		**>8.35 (5/5) p=0.0079**	**>8.35 (5/5) p=0.0079**	**>8.35 (5/5) p=0.0079**
		Joint Tape Paper	**>8.72 (5/5) p=0.0079**	**>8.72 (5/5) p=0.0079**		**>8.72 (5/5) p=0.0079**	**>8.72 (5/5) p=0.0079**	**>8.72 (5/5) p=0.0079**
Trial 2a (500 ppmv)	*B. anthracis* spores	Carpet	**3.23 (2.23, 4.24) p=0.0003**	**>5.36 (1/5) p=0.0079**		**>8.12 (5/5) p=0.0079**	**>8.12 (5/5) p=0.0079**	**>8.12 (5/5) p=0.0079**
		Concrete	**>7.72 (4/5) p=0.0079**	**>8.28 (5/5) p=0.0079**		**>8.28 (5/5) p=0.0079**	**>8.28 (5/5) p=0.0079**	**>8.28 (5/5) p=0.0079**
		Galvanized Metal	**>8.17 (5/5) p=0.0079**	**>8.17 (5/5) p=0.0079**		**>8.17 (5/5) p=0.0079**	**>8.17 (5/5) p=0.0079**	**>8.17 (5/5) p=0.0079**
		Laminate	**>8.35 (5/5) p=0.0079**	**>8.35 (5/5) p=0.0079**		**>8.35 (5/5) p=0.0079**	**>8.35 (5/5) p=0.0079**	**>8.35 (5/5) p=0.0079**
Trial 2b (200-250 ppmv)	*B. anthracis* spores	Carpet	**1.27 (1.17, 1.37) p<0.0001**	**>5.06 (2/5) p=0.0079**		**>6.39 (4/5) p=0.0079**	**>6.12 (3/5) p=0.0079**	**>6.42 (4/5) p=0.0079**
		Concrete	**>5.28 (2/5) p=0.0079**	**>6.52 (4/5) p=0.0079**		**>6.83 (5/5) p=0.0079**	**>6.83 (5/5) p=0.0079**	**>6.83 (5/5) p=0.0079**
		Galvanized Metal	**>6.72 (5/5) p=0.0079**	**>6.72 (5/5) p=0.0079**		**>6.72 (5/5) p=0.0079**	**>6.72 (5/5) p=0.0079**	**>6.72 (5/5) p=0.0079**
		Laminate	**>6.14 (4/5) p=0.0079**	**>6.50 (5/5) p=0.0079**		**>6.50 (5/5) p=0.0079**	**>6.50 (5/5) p=0.0079**	**>6.50 (5/5) p=0.0079**
Trial 3a (500 ppmv)	*B. anthracis* spores	Ceiling Tile	**>5.87 (5/5) p=0.0079**	**>5.87 (5/5) p=0.0079**		**>5.87 (5/5) p=0.0079**	**>5.87 (5/5) p=0.0079**	**>5.87 (5/5) p=0.0079**
		Glass	**>5.16 (2/5) p=0.0079**	**>6.67 (5/5) p=0.0079**		**>6.67 (5/5) p=0.0079**	**>6.67 (5/5) p=0.0079**	**>6.67 (5/5) p=0.0079**
		Wood	**1.93 (1.24, 2.62) p=0.0023**	**>2.95 (1/5) p=0.0079**		**>3.49 (1/5) p=0.0079**	**>5.72 (5/5) p=0.0079**	**>5.72 (5/5) p=0.0079**
Trial 3b (200-250 ppmv)	*B. anthracis* spores	Ceiling Tile	**>4.86 (2/5) p=0.0079**	**>5.20 (3/5) p=0.0079**		**>5.87 (5/5) p=0.0079**	**>5.87 (5/5) p=0.0079**	**>5.87 (5/5) p=0.0079**
		Glass	**>6.11 (4/5) p=0.0079**	**>6.05 (4/5) p=0.0079**		**>6.48 (5/5) p=0.0079**	**>6.48 (5/5) p=0.0079**	**>6.48 (5/5) p=0.0079**
		Wood	**1.35 (0.98, 1.72) p=0.0007**	**2.14 (1.42, 2.87) p=0.0022**		**2.13 (2.00, 2.26) p<0.0001**	**>3.51 (2/5) p=0.0079**	**>3.31 (2/5) p=0.0079**
Trial 4a (200-250 ppmv)	*Y. pestis*	Aluminum			**>4.51 (5/5) p=0.0079**	**>4.62 (5/5) p=0.0079**		
		Keyboard			**>2.59 (5/5) p=0.0079**	**>1.80 (5/5) p=0.0079**		

Trials	Agent	Material	Mean Log Reduction (95% CI) and P-value* Or Mean Log Reduction (# of treated coupons with zero recovery/# of treated coupons) and P-value†					
			30 min	**60 min**	**90 min**	**120 min**	**240 min**	**Gassing + 4hr**
Trial 4b (500 ppmv)	*Y. pestis*	Aluminum	**>4.22 (5/5) p=0.0079**					
		Keyboard	**>4.69 (5/5) p=0.0079**					
Trial 5a (200-250 ppmv)	*F. tularensis*	Aluminum			**>6.30 (5/5) p=0.0079**			
		Keyboard			**>5.73 (5/5) p=0.0079**			
Trial 5b (500 ppmv)	*F. tularensis*	Aluminum	**>6.63 (5/5) p=0.0079**					
		Keyboard	**>5.58 (5/5) p=0.0079**					
Trial 6a (200-250 ppmv)	*B. suis*	Aluminum			**>6.21 (1/5) p=0.0079**	**>7.46 (5/5) p=0.0079**		
		Carpet			**>4.96 (5/5) p=0.0079**	**>6.29 (5/5) p=0.0079**		
		Keyboard			**5.03 (3.94, 6.13) p=0.0004**	**>6.51 (4/5) p=0.0079**		
		Painted Joint Tape			**>5.47 (5/5) p=0.0079**	**>6.05 (4/5) p=0.0079**		
Trial 6b (500 ppmv)	*B. suis*	Aluminum	**4.29 (4.16, 4.42) p<0.0001**	**>5.69 (1/5) p=0.0079**	**>7.70 (5/5) p=0.0079**			
		Carpet	**>6.49 (5/5) p=0.0079**	**>5.79 (5/5) p=0.0079**				
		Keyboard	**4.34 (4.21, 4.48) p<0.0001**	**>6.25 (2/5) p=0.0079**	**>7.69 (5/5) p=0.0079**			
		Painted Joint Tape	**>4.22 (5/5) p=0.0079**	**>4.56 (5/5) p=0.0079**				
Trial 7a (200-250 ppmv)	Vaccinia Virus	Aluminum	**5.20 (4.92, 5.47) p<0.0001**	**5.05 (4.95, 5.16) p<0.0001**		**4.68 (4.52, 4.84) p<0.0001**		
		Carpet	**>3.09 (5/5) p=0.0079**					
		Keyboard	**4.22 (4.02, 4.43) p<0.0001**	**>4.92 (5/5) p=0.0079**				
		Painted Joint Tape	**>4.22 (5/5) p=0.0079**					
Trial 7b (500 ppmv)	Vaccinia Virus	Aluminum	**4.54 (4.21, 4.88) p<0.0001**	**5.01 (4.95, 5.07) p<0.0001**				
		Keyboard	**4.38 (4.21, 4.55) p<0.0001**	**>5.66 (5/5) p=0.0079**				

* Mean log reduction is the mean of the base-10 logarithm of recovered agent from the control coupons minus the mean of the base-10 logarithm of recovered agent from the treated coupons. A 95 % CI for the difference is shown in parentheses. A p-value is provided for the probability that the control and treatment recoveries are the same. The p-value is from the two sample t-test with Satterthwaite's method to allow for potentially different variances in the two groups. p-Values less than 0.05 denote less than 1 in 20 chance that a difference as large as or larger than observed would occur by chance if the control and treatment means were truly identical. Comparisons with p-values less than 0.05 (statistically significant at the 0.05 level) are bolded.

† One or more of the treatment coupons had no recovered agent. The mean log reduction of the form ">X" is calculated as the mean of the base-10 logarithm of recovered agent from the control coupons minus the mean of the base-10 logarithm of recovered agent from the treated coupons except that "zero recovery" coupons have a substituted recovered value of "1" (base-10 log is 0). Since the log becomes an increasingly negative value below 1 and is undefined at 0, this substitution is necessary and results in a lower bound on the mean log difference, as indicated by the ">". The number of "zero recovery" treatment coupons and the total number of treatment coupons is shown in parentheses. The p-value is from the non-parametric Kolmogorov-Smirnov test. p-Values less than 0.05 denote less than 1 in 20 chance that results as different as or more different than observed would occur by chance if the distribution of the control and treatment recoveries were truly identical. Comparisons with p-values less than 0.05 (statistically significant at the 0.05 level) are bolded.

6.0 Performance Summary

The persistence of the biological agents (*B. suis*, *F. tularensis*, vaccinia virus, and *Y. pestis*) on various building materials varied by organism and material type. All biological agents persisted at least 7 days (168 hr) on at least one building material. However, the null hypothesis that, given an equivalent application of biological agent, the amount of biological agent on the coupons was constant over time, was rejected. For many combinations of biological agent and material, significant loss of viable biological agent occurred within 2 hr. Significant loss of viable biological agent was observed for all combinations of biological agent and material within three days.

At the tested conditions, none of the decontamination technologies evaluated eliminated the recovery of every tested biological agent from every tested material. The results of the fumigation testing are summarized in Tables 6-1, 6-2, 6-3 and 6-4. However, the null hypothesis that there was no difference in the decontamination efficacy using the treatment compared to the positive controls was rejected (except in the cases of high loss of viable biological agent from positive control coupons). Significant loss of viable biological agent compared to the positive controls was observed for all biological agents using all four decontamination technologies. In some cases the biological agent on certain materials exhibited a high loss of recoverable biological agent from the positive control coupons (e.g., *F. tularensis* on carpet at 40% RH). In these cases, even though little or no viable biological agent was recovered from the test coupons, the loss of viable biological agent from the positive control coupons resulted in a low base for statistical comparison and the null hypothesis could not be rejected – there were no difference in the high level of decontamination efficacy using the treatment compared to the high level of loss of biological agent from the positive controls. The combined effect of loss of viability over time (without decontamination treatment) and decontamination efficacy determines the overall effectiveness of a treatment.

Sabre ClO_2 fumigation was generally more efficacious at 75% RH than at lower RH (e.g., 40%).

Sabre ClO_2 fumigation at 3,000 ppmv and 75% RH resulted in no viable *B. anthracis* spores being recovered from keyboard (40-min contact time), carpet (90-min contact time), or joint tape (90-min contact time). At 40% RH, *B. anthracis* spores remained viable on carpet and joint tape after 90 min of exposure to 3,000 ppmv ClO_2. Viable *B. anthracis* spores were recovered from aluminum following all tests with 3,000 ppmv ClO_2, although the mean amount of recovered spores was generally multiple logs lower at 75% RH than 40% RH.

Sabre ClO_2 fumigation at 50-100 ppmv and 75% RH resulted in no viable *B. suis* being recovered from aluminum, carpet or joint tape after a 60-min contact time. However, at 50-100 ppmv ClO_2 with a 60 min contact time and 40% RH, viable *B. suis* was recovered from aluminum, carpet, and joint tape at greater than 10^3 CFUs/coupon. *B. suis* was recovered from keyboard following all tests with 50-100 ppmv ClO_2, although the mean amount of recovered agent was generally lower at 75% RH than 40% RH.

Sabre ClO_2 fumigation at 50-100 ppmv and 75% RH resulted in no viable *F. tularensis* being recovered from aluminum or keyboard after a 120-min contact time. At 50-100 ppmv ClO_2 and 40% RH, viable *F. tularensis* was recovered from aluminum and keyboard at levels greater than 10^5 CFUs/coupon. No viable *F. tularensis* was recovered from any of the tests with carpet or joint tape; natural degradation of *F. tularensis* may have been an important contributing factor (especially at 40% RH) as the associated positive controls demonstrated relatively low recoveries.

Vaccinia virus generally remained viable on keyboard for all tests conducted with Sabre ClO_2. No viable vaccinia virus was recovered from aluminum, carpet, or joint tape with 50 – 100 ppmv ClO_2 at 75% RH following a 30-min contact time. Comparable testing at 40% RH resulted in viable vaccinia virus being detected at greater than 10^4 PFUs/coupon.

Tests with Sabre ClO_2 fumigation at 50-100 ppmv and 40% RH or 75% RH resulted in no viable *Y. pestis* recovered from any of the tests with aluminum, keyboard, carpet, or joint tape; natural degradation of *Y. pestis* may have been an important contributing factor as the associated positive controls generally demonstrated low recoveries.

BIOQUELL Clarus C HP with a fumigation cycle of 10 min at 8 g/min, dwell at 0.8 g/min with a 180-min contact time resulted in no *B. anthracis* spores being recovered from laminate, ductwork, glass, and ceiling tile; viable spores were recovered from carpet, concrete, and wood under these conditions.

BIOQUELL Clarus C HP with a fumigation cycle of 10 min at 8 g/min, dwell at 0.8 g/min with a 180-min contact time resulted in no viable *B. suis*, vaccinia virus, or *Y. pestis* being recovered from any of the materials tested (aluminum, keyboard, carpet, glass [vaccinia only], and joint tape).

BIOQUELL Clarus S HP with a fumigation cycle of 50 mL HP injected over 20 min (initial RH: 40%-50%) with a 75-min contact time resulted in no *B. anthracis* spores being recovered from aluminum, keyboard, and joint tape; viable spores were recovered from carpet under these conditions.

BIOQUELL Clarus S HP with a fumigation cycle of 15 mL injected over 15 min (65% initial RH) with a 15 or 30-min contact time resulted in no viable *F. tularensis* being recovered from the materials tested (aluminum, keyboard, carpet, and joint tape).

BIOQUELL Clarus S HP with a fumigation cycle of 15 mL injected over 15 min (65% initial RH) with a 60-min contact time resulted in viable *B. suis* and *Y. pestis* being recovered from the aluminum, keyboard, and carpet, but not from joint tape.

STERIS VHP® HP fumigation with a nominal concentration of 500 ppmv and a 120-min contact time resulted in no *B. anthracis* spores being recovered from any material tested (except wood). Materials tested included aluminum, keyboard, joint tape, carpet, laminate, ductwork, ceiling tile, glass, concrete. No viable spores were recovered from wood after a 240-min contact time under these conditions (~500 ppmv).

STERIS VHP® HP fumigation with the 500 ppmv fumigation cycle resulted in no viable *B. suis* from carpet or joint tape (after a 30-min contact time) or from aluminum or keyboard (after a 90-min contact time). After a 120-min contact time in the 200-250 ppmv HP fumigation cycle, viable *B. suis* was recovered from keyboard and joint tape, but not aluminum and carpet.

STERIS VHP® HP fumigation with the 200-250 ppmv fumigation cycle for 90 min and fumigation with the 500 ppmv fumigation cycle for 30 min resulted in no viable *F. tularensis* or *Y. pestis* recovered from aluminum or keyboard (carpet and joint tape were not tested).

STERIS VHP® HP fumigation with the 200-250 ppmv fumigation cycle for 30 min resulted in no viable vaccinia virus being recovered from carpet or joint tape (but vaccinia virus was recovered from aluminum and keyboard). When the contact time was increased to 60 min, vaccinia virus was no longer recovered from keyboard. However, vaccinia virus continued to be recovered from aluminum even when the contact time was increased to 120 min (at the 200-250 ppmv fumigation cycle). Following a 60-min exposure to the 500 ppmv fumigation cycle, viable vaccinia virus was recovered from aluminum but not keyboard.

Biological indicators were used in parallel with the biological agent decontamination testing. The biological agents that were used were *B. atrophaeus* spores (nominally 10^6 spores) on steel in Tyvek® packaging, for the Sabre ClO_2 fumigation testing and *G. stearothermophilus* (nominally 1 x 10^6 spores) on stainless steel in Tyvek® packaging for the three HP technologies. The results from qualitative evaluation of the biological indicators did not correlate consistently with the results from quantitative evaluation of viable biological agent remaining on coupons of various materials. For example, the *B. atrophaeus* biological indicators used for the Sabre ClO_2 fumigation were all positive for growth at the 180-min contact time (the longest time tested), indicative of incomplete kills. The biological indicators (on steel) are consistent with *B. anthracis* on aluminum which retained viable spores under all treatment conditions. However, no *B. anthracis* spores were recovered from keyboard or carpet under the same conditions. In contrast, the *G. stearothermophilus* biological indicators were negative for growth, indicating complete kills, after fumigation treatments in which viable *B. anthracis*, *B. suis*, and vaccinia virus were recovered from some materials. For these hardy biological agents, observations of no growth of biological indicators cannot be assumed to correlate to no viable biological agent remaining on any material.

Table 6-1. Summary of Sabre ClO_2 Fumigation

Decontamination Method	Biological Agent	Aluminum	Keyboard	Carpet	Joint Tape
Sabre ClO_2 3,000 ppmv, **40%RH**	*B. anthracis* spores	Viable spores @ 180 min	0 CFUs @ 20 min	0 CFUs @ 180 min	Viable spores @ 180 min
Sabre ClO_2 3,000 ppmv, **75%RH**	*B. anthracis* spores	Viable spores @ 180 min	0 CFUs @ 40 min	0 CFUs @ 90 min	0 CFUs @ 90 min
SSabre ClO_2 50-100 ppmv, **40%RH**	*B. suis*	Viable bacteria @ 120 min	Viable bacteria @ 120 min	0 CFUs @ 120 min	0 CFUs @ 120 min
Sabre ClO_2 50-100 ppmv, **60%RH**	*B. suis*	Viable bacteria @ 120 min	Viable bacteria @ 120 min	0 CFUs @ 120 min	0 CFUs @ 30 min
Sabre ClO_2 50-100 ppmv, **75%RH**	*B. suis*	0 CFUs @ 60 min	Viable bacteria @ 120 min	0 CFUs @ 30 min	0 CFUs @ 30 min
Sabre ClO_2 50-100 ppmv, **40%RH**	*F. tularensis*	Viable bacteria @ 120 min	Viable bacteria @ 120 min	0 CFUs @ 30 min	0 CFUs @ 30 min
Sabre ClO_2 50-100 ppmv, **75%RH**	*F .tularensis*	0 CFUs @ 120 min	0 CFUs @ 120 min	0 CFUs @ 30 min	0 CFUs @ 30 min
Sabre ClO_2 50-100 ppmv, **40%RH**	Vaccinia virus	Viable virus @ 120 min	Viable virus @ 120 min	Viable virus @ 120 min	Viable virus @ 120 min
Sabre ClO_2 50-100 ppmv, **60%RH**	Vaccinia virus	Mixed results @ 120 min	Mixed results @ 120 min	0 PFUs @ 120 min	0 PFUs @ 120 min
Sabre ClO_2 50-100 ppmv, **75%RH**	Vaccinia virus	0 PFUs @ 30 min	Viable virus @ 120 min	0 PFUs @ 30 min	0 PFUs @ 30 min
Sabre ClO_2 50-100 ppmv, **40%RH**	*Y. pestis*	0 CFUs @ 30 min	0 CFUs @ 30 min	0 CFUs @ 30 min	0 CFUs @ 30 min
Sabre ClO_2 50-100 ppmv, **75%RH**	*Y. pestis*	0 CFUs @ 30 min	0 CFUs @ 30 min	0 CFUs @ 30 min	0 CFUs @ 30 min

Table 6-2. Summary of BIOQUELL Clarus C HP Fumigation

Decontamination Method	Biological Agent	Aluminum	Keyboard	Carpet	Joint Tape
BIOQUELL Clarus C HP injection for 10 min at 8 g/min; dwell at 0.8 g/min	*B. anthracis* spores	--	--	0 CFUs @ 180 min	--
BIOQUELL Clarus C HP injection for 5 min at 8 g/min; dwell at 0.8 g/min	*B. anthracis* spores	--	--	Viable bacteria @ 180 min	--
BIOQUELL Clarus C HP injection for 10 min at 8 g/min; dwell at 0.8 g/min*	*B. anthracis* spores	--	--	Viable bacteria @ 180 min	--
BIOQUELL Clarus C HP injection for 10 min at 8 g/min; dwell at 0.8 g/min	*B. suis*	0 CFUs @ 180 min	0 CFUs @ 180 min	0 CFUs @ 180 min	0 CFUs @ 180 min
BIOQUELL Clarus C HP injection for 10 min at 8 g/min; dwell at 0.8 g/min†	Vaccinia virus	0 PFUs @ 180 min	0 PFUs @ 180 min	0 PFUs @ 180 min	0 PFUs @ 180 min
BIOQUELL Clarus C HP injection for 10 min at 8 g/min; dwell at 0.8 g/min	*Y. pestis*	0 CFUs @ 180 min	0 CFUs @ 180 min	0 CFUs @ 180 min	0 CFUs @ 180 min

* No spores were recovered from laminate, ductwork, glass, and ceiling tile under these conditions (0 CFUs @ 180 min); viable spores were recovered from carpet, concrete, and wood under these conditions (180 min).
† No viable bacteria were recovered from glass under these conditions (0 CFUs @ 180 min).
-- Not tested.

Table 6-3. Summary of BIOQUELL Clarus S HP Fumigation

Decontamination Method	Biological Agent	Aluminum	Keyboard	Carpet	Joint Tape
BIOQUELL Clarus S 3 x ~15 mL injection, **45%RH**	*B. anthracis* spores	--	--	Viable bacteria @ 192 min	--
BIOQUELL Clarus S 15 mL injection, **45%RH**	*B. anthracis* spores	--	0 CFUs @ 30 min	Viable bacteria @ 60 min	--
BIOQUELL Clarus S 50 mL injection, **45%RH**	*B. anthracis* spores	0 CFUs @75 min	0 CFUs @75 min	Viable bacteria @ 75 min	0 CFUs @75 min
BIOQUELL Clarus S 15 mL injection, **45%RH**	*B. suis*	Viable bacteria @ 30 min	0 CFUs @ 30 min	Viable bacteria @ 30 min	Viable bacteria @ 30 min
BIOQUELL Clarus S 15 mL injection, **65%RH**	*B. suis*	Viable bacteria @ 60 min	Viable bacteria @ 60 min	Viable bacteria @ 60 min	0 CFUs @ 60 min
BIOQUELL Clarus S 15 mL injection, **45%RH**	*F. tularensis*	0 CFU @ 15 min	0 CFUs @ 15 min	0 CFUs @ 15 min	0 CFUs @ 15 min
BIOQUELL Clarus S 15 mL injection, **65%RH**	*F. tularensis*	0 CFUs @ 30 min	0 CFUs @ 30 min	0 CFUs @ 15 min	0 CFUs @ 15 min
BIOQUELL Clarus S 15 mL injection, **45%RH**	*Y. pestis*	0 CFUs @ 30 min	Viable bacteria @ 30 min	Viable bacteria @ 30 min	Viable bacteria @ 30 min
BIOQUELL Clarus S 15 mL injection, **65%RH**	*Y. pestis*	Viable bacteria @ 60 min	Viable bacteria @ 60 min	Viable bacteria @ 60 min	0 CFUs @ 60 min

-- Not tested.

Table 6-4. Summary of STERIS VHP® HP Fumigation

Decontamination Method	Biological Agent	Aluminum	Keyboard	Carpet	Joint Tape
STERIS VHP® 200 - 250 ppmv*	*B. anthracis* spores	--	--	Viable bacteria @ full cycle 240 min	--
STERIS VHP® 500 ppmv†	*B. anthracis* spores	0 CFUs @ 30 min	0 CFUs @ 30 min	0 CFUs @ 120 min	0 CFUs @ 30 min
STERIS VHP® 200 - 250 ppmv	*B. suis*	0 CFUs @ 120 min	Viable bacteria @ 120 min	0 CFUs @ 90 min	0 CFUs @ 90 min; 2nd run, viable bacteria @ 120 min
STERIS VHP® 500 ppmv	*B. suis*	0 CFUs @ 90 min	0 CFUs @ 90 min	0 CFUs @ 30 min	0 CFUs @ 30 min
STERIS VHP® 200 - 250 ppmv	*F. tularensis*	0 CFUs @ 90 min	0 CFUs @ 90 min	--	--
STERIS VHP® 500 ppmv	*F. tularensis*	0 CFUs @ 30 min	0 CFUs @ 30 min	--	--
STERIS VHP® 200 - 250 ppmv	Vaccinia virus	Viable virus @ 120 min	0 PFUs @ 60 min	0 PFUs @ 30 min	0 PFUs @ 30 min
STERIS VHP® 500 ppmv	Vaccinia virus	Viable virus @ 60 min	0 PFUs @ 60 min	--	--
STERIS VHP® 200 - 250 ppmv	*Y. pestis*	0 CFUs @ 90 min	0 CFUs @ 90 min	--	--
STERIS VHP® 500 ppmv	*Y. pestis*	0 CFUs @ 30 min	0 CFUs @ 30 min	--	--

* No spores were recovered from ductwork after a 30-min contact time, from laminate after a 60-min contact time; concrete, glass or ceiling tile after a 120-min contact time under these conditions (200 - 250 ppmv); viable spores were recovered from wood after 240-min contact time under these conditions.
† No spores were recovered from laminate, ductwork, or ceiling tile after a 30-min contact time, from glass or concrete after a 60-min contact time; or from wood after a 240-min contact time at this condition (500 ppmv).
-- Not tested.

7.0 References

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Appendix A
Specific Deviations of Tests Not Repeated

Method Demonstration - Biological Agent Recovery Testing:

B. anthracis spores target application range: 7.50×10^6 - 1.25×10^7 CFUs
Actual application: 9.17×10^7 - 9.87×10^7 CFUs
(Inadvertently used an application that was one log too high; results do not impact test results.)

Persistence Testing with *F. tularensis*:

Target positive control % recovery range: ≥10% - ≤120%
Actual recovery from carpet: 169%
Actual recovery from keyboard keys: 121%

Persistence Testing with Vaccinia Virus:

Target positive control recovery criteria: ≥1 x 10^5 PFUs and CV ≤25%
Actual CV associated with joint tape: 25.5%

Persistence Testing with *Y. pestis*:

Target positive control % recovery range: ≥10% - ≤120%
Actual recovery from aluminum: 224%
Actual recovery from keyboard keys: 136% and 288%
Actual recovery from carpet: 329%
Actual recovery from joint tape: 150%

Sabre Fumigation Testing with *B. anthracis* spores:

Target positive control % recovery range: ≥10% - ≤120%
Actual recovery from aluminum at 75% RH: 109.5% - 138.2%
Actual recovery from keyboard keys at 75% RH: 232.5% - 433.3%
Actual recovery from joint tape at 75% RH: 66.2% - 133.7%

B. anthracis spores target application range: 7.50×10^6 - 1.25×10^7 CFUs
Actual application at 75% RH: 3.83×10^6 - 5.73×10^6 CFUs

Sabre Fumigation Testing with *B. suis*:

Target positive control % recovery range: ≥10% - ≤120%
Actual recovery from joint tape (40% RH, 60% RH, and 75%RH): 0.01% - 0.88%
Actual recovery from aluminum at 75% RH: 1557%

Sabre Fumigation Testing with *F. tularensis*:

F. tularensis target application range: 1.0×10^6 - 1.0×10^8 CFUs
Actual application: 5.17×10^7 - 1.15×10^8 CFUs

Target positive control % recovery range: ≥10% - ≤120%
Actual recovery from joint tape (40% RH and 75% RH): 0.0% - 1.09%
Actual recovery from aluminum at 40% RH: 4.07%

Sabre Fumigation Testing with vaccinia virus:

Target positive control recovery criteria: ≥1 x 10^5 PFUs and CV ≤25%
Actual CV from aluminum (40% RH, 60% RH, and 75%RH): 43.7% - 79.6%
Actual CV from keyboard keys (40% RH, 60% RH, and 75%RH): 20.4% - 74.5%
Actual CV from carpet (40% RH, 60% RH, and 75%RH): 36.6% - 112.2%
Actual CV from joint tape (40% RH, 60% RH, and 75%RH): 25.9% - 53.3%

Sabre Fumigation Testing with *Y. pestis*:

Target positive control % recovery range: ≥10% - ≤120%
Actual recovery from aluminum (40% RH and 75% RH): 0.00% - 0.16%
Actual recovery from keyboard keys (40% RH and 75% RH): 0.03% - 0.08%
Actual recovery from joint tape (40% RH and 75% RH): 0.00%

BIOQUELL Clarus C Fumigation Testing with *B. anthracis* spores:

Target positive control % recovery range: ≥10% - ≤120%
Actual recovery from wood: 4.86%
Actual recovery from ceiling tile: 6.17%

BIOQUELL Clarus C Fumigation Testing with vaccinia virus:

Target positive control recovery criteria: ≥1 x 10^5 PFUs and CV ≤25%
Actual recovery from carpet: 3.45 x 10^4 PFUs and CV 56.3%
Actual recovery from keyboard keys: CV 74.1%
Actual recovery from joint tape: CV 54.9%
Actual recovery from aluminum: CV 79.5%
Actual recovery from glass: CV 26.1%

BIOQUELL Clarus C Fumigation Testing with *Y. pestis*:

Target positive control % recovery range: ≥10% - ≤120%
Actual recovery from keyboard keys: 4.12%
Actual recovery from joint tape: 1.82%
Actual recovery from aluminum: 0.80%

BIOQUELL Clarus S Fumigation Testing with *B. anthracis* spores:

B. anthracis spores target application range: 7.50 x 10^6 - 1.25 x 10^7 CFUs
Actual application: 2.33 x 10^6 - 8.40 x 10^6 CFUs

BIOQUELL Clarus S Fumigation Testing with *B. suis*:

B. suis target application range: 1.0 x 10^6 - 1.0 x 10^8 CFUs
Actual application: 3.10 x 10^7 - 1.77 x 10^8 CFUs

BIOQUELL Clarus S Fumigation Testing with *F. tularensis*:

Target positive control % recovery range: ≥10% - ≤120%
Actual recovery from keyboard keys: 1.19%
Actual recovery from joint tape: 0.02%
Actual recovery from aluminum: 0.06%

BIOQUELL Clarus S Fumigation Testing with *Y. pestis*:

Target positive control % recovery range: ≥10% - ≤120%
Actual recovery from keyboard keys: 0.10%
Actual recovery from joint tape: 0.63%
Actual recovery from aluminum: 0.53%

STERIS VHP® Fumigation Testing with *B. anthracis* spores:

B. anthracis spores target application range: 7.50 x 10^6 - 1.25 x 10^7 CFUs
Actual application: 6.93 x 10^6 - 4.87 x 10^8 CFUs

Target positive control % recovery range: ≥10% - ≤120%
Actual recovery from carpet (Trial 1): 158.1%
Actual recovery from aluminum (Trial 1): 149.9%
Actual recovery from wood (Trials 3a and 3b): 3.52% - 5.60%
Actual recovery from ceiling tile (Trials 3a and 3b): 7.47% - 7.66%

STERIS VHP® Fumigation Testing with *B. suis*:

Target positive control % recovery range: ≥10% - ≤120%
Actual recovery from joint tape: 0.06% - 2.11%

STERIS VHP® Fumigation Testing with *F. tularensis*:

F. tularensis target application range: 1.0 x 10^6 - 1.0 x 10^8 CFUs
Actual application: 2.53 x 10^7 - 4.70 x 10^8 CFUs
Target positive control % recovery range: ≥10% - ≤120%
Actual recovery from keyboard keys: 0.08% - 0.39%
Actual recovery from aluminum: 3.18% - 5.31%

STERIS VHP® Fumigation Testing with vaccinia virus:

Target positive control recovery criteria: ≥1 x 10^5 PFUs and CV ≤25%
Actual recovery from carpet: 6.63 x 10^4 PFUs and CV 42.31%
Actual recovery from keyboard: CV 25.5% - 26.8%
Actual recovery from joint tape: 1.94 x 10^4 PFUs and CV 23.50%

STERIS VHP® Fumigation Testing with *Y. pestis*:

Target positive control % recovery range: ≥10% - ≤120%
Actual recovery from keyboard keys: 0.01% - 0.95%
Actual recovery from aluminum: 3.30% - 5.45%

Further, during persistence testing with *B. suis,* contamination of blanks occurred for a limited number of trials.

Blank acceptance criteria: no observed CFUs
Actual results: 2.30 x 10^2 CFUs/coupon on aluminum at 0 hr, 6.70 x 10^1 CFUs/coupon on aluminum at 2 hr, and 3.00 x 10^1 CFUs/coupon on keyboard keys at 3 days.

The biological agent contamination issue was limited in scope and was not expected to affect the overall results of the associated persistence test results. Once the contamination was noted, fumigation of the hoods, increased physical separation of the coupons, and discussions with technical staff to raise awareness of the problem were used to successfully eliminate the contamination issue.

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